Vol. 30, No. 1 May, 1943

THE ANNALS OF APPLIED BIOLOGY

EDITED FOR THE ASSOCIATION OF APPLIED BIOLOGISTS

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CAMBRIDGE UNIVERSITY PRESS

LONDON: BENTLEY HOUSE

CHICAGO: The University of Chicago Press
(Agents for the United States)

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The nutrition of the carrot

BY R. M. WOODMAN

Horticultural Research Station, School of Agriculture, Cambridge University

In sand-culture experiments with carrots, it was shown that a moderate quantity only of nitrogen was necessary for optimum growth compared with, for example, the turnip root. A moderate amount of (available) phosphorus was also sufficient for this purpose, as with the turnip, but contrary to experience with lettuce roots and tops. The greatest concentration of potassium applied, however, was probably the best for root development. Deficiency of phosphorus caused bronzing of the leaves, and absence of potassium, serious scorch; absence of boron resulted in a small, immature plant. Initial field trials on an old river gravel during experiments throughout the last 7 years indicated that dung gave no advantage over artificials, that artificials were possibly not needed on dunged land or land in good heart, and that land out of good heart, undunged and unfertilized throughout the 7 years, responded well to artificials, particularly phosphate and potash. The incidence of carrot fly, according to preliminary experiments, seemed to depend greatly on the nutrition of the carrots.

This paper contains results of sand cultures and field trials with carrots; the variety was Primo, of Watkins and Simpson.

SAND CULTURES OF CARROTS

The general methods employed have been described (Woodman, 1940). Concentrations of certain elements present in the nutrient solutions are given in Table 1, where variations in the concentration of any element are given in heavy type. The numerical data obtained have also been discussed under the above reference.

TABLE 1. *Concentration (p.p.m.) of important elements in the culture solutions*

Solution ...	N	P	K	B	S	Na
			Nitrogen experiment			
A	**65·92**	8·19	22·44	0·0681	13·11	**120·45**
B	**32·96**	8·19	22·44	0·0681	13·11	**66·31**
C	**16·48**	8·19	22·44	0·0681	13·11	**39·23**
D	**8·24**	8·19	22·44	0·0681	13·11	**25·69**
E	**4·12**	8·19	22·44	0·0681	13·11	**18·92**
F	**0·41**	8·19	22·44	0·0681	13·11	**12·83**
G	**Nil**	8·19	22·44	0·0681	13·11	**12·15**
			Phosphorus experiment			
A	32·96*	**16·38**	22·44	0·0681	13·11	**78·46***
B	32·96	**8·19**	22·44	0·0681	13·11	**66·31**
C	32·96	**6·14**	22·44	0·0681	13·11	**63·27**
D	32·96	**4·09**	22·44	0·0681	13·11	**60·23**
E	32·96	**2·05**	22·44	0·0681	13·11	**57·20**
F	32·96	**1·02**	22·44	0·0681	13·11	**55·68**
G	32·96	**0·10**	22·44	0·0681	13·11	**54·31**
			Potassium and boron experiments			
A	32·96†	8·19	**22·44**	0·0681	**13·11**	66·31†
B	32·96	8·19	22·44	**Nil**	13·11	66·31
C	32·96	8·19	**11·22**	0·0681	**8·51**	66·31
D	32·96	8·19	**5·61**	0·0681	**6·21**	66·31
E	32·96	8·19	**2·81**	0·0681	**5·06**	66·31
F	32·96	8·19	**0·28**	0·0681	**4·03**	66·31
G	32·96	8·19	**Nil**	0·0681	**3·91**	66·31

In addition, there were present in all the solutions, 1·01 p.p.m. of iron as ferrous sulphate, 2·53 p.p.m. of magnesium as the sulphate, and 13·55 p.p.m. of calcium as chloride; the sulphur of the first two is contained in the amounts quoted in the table. Minor elements were also present in the following proportions: 0·1847 p.p.m. of manganese as the sulphate (tetrahydrate); 0·0682 p.p.m. of zinc as the sulphate; 0·0095 p.p.m. of aluminium as ammonium alum; 0·0164 p.p.m. of the ammonium radicle $(NH_4)^{\cdot}$, partly as the alum above, and partly as ammonium sulphate; and 0·0153 p.p.m. of copper as the sulphate. The elements combined with these small amounts of the minor elements in the above salts were neglected in compiling the table.

* Because this amount of N appeared to be prejudicial to growth, it was reduced, from 14 Feb. 1941, to 12 p.p.m. for all solutions. The Na was thus reduced throughout by 13·54 p.p.m.

Similar remarks apply. The reduction, on 27 Sept. 1939, was to 12·36 p.p.m. of N; the corresponding reduction of K for all solutions was thus by 33·85 p.p.m.

OBSERVATIONS AND DISCUSSIONS OF RESULTS

Nitrogen experiment

The number of waxed plant-pots was 112, in a randomized block of the seven treatments replicated sixteen times. Seed sown, 27 Sept. 1939; germinated by 9 Oct.; singling, 24 Oct.; and cultures harvested, 5 Feb. 1940. Each culture received 18 l. of its appropriate solution in lots of 250 c.c. about three times a week.

On 24 Oct. 1939, all seedlings were the same green colour, and those receiving most nitrogen, solution A, were the largest. By 28 Nov. some cultures with treatments A and B showed a slight tendency to chlorosis, which persisted for over a month. At harvest, 5 Feb. 1940, there was no serious difference in the cultures except in size, and all signs of chlorosis had disappeared.

TABLE 2. *Summaries of results for nitrogen experiment. Weights in g.*

Description of data		Treatment mean for A	B	C	D	E	F	G	Mean of all results	S.E.
Tops, FW	SSS	3·80	3·03	3·70	2·98	2·17	0·52	0·07	2·32	0·244
		A>B=D>E>F=G; A=C>E; B=C=D								
Roots, FW	SSS	9·04	8·52	11·28	11·12	8·78	1·63	0·22	7·23	0·949
		A=B=C=D=E>F=G								
Whole plants, FW	SSS	12·88	11·55	14·98	14·10	10·95	2·15	0·29	9·56	1·118
		A=C=D>F=G; C>B=E; A=B=D=E>F=G								
Tops, DW	SS	0·61	0·53	0·62	0·48	0·34	0·09	0·01	0·38	0·127
		A=B=C=D>F=G; A=B=C=D=E; E=F=G								
Roots, DW	SSS	0·85	0·78	1·05	1·02	0·84	0·17	0·02	0·68	0·078
		A=C=D=E>F=G; C=D>B; A=B=E>F=G								
Whole plants, DW	SSS	1·47	1·31	1·67	1·49	1·18	0·26	0·03	1·06	0·107
		A=C=D>F=G; C>B=E; A=B=D=E>F=G								
Top/root, FW	SS	0·47	0·38	0·36	0·31	0·26	0·36	0·46	0·37	0·042
		A=G>D=E; A=B=C=F=G; B=C=D=E=F								
Top/root, DW	SS	0·80	0·70	0·64	0·53	0·42	0·58	0·76	0·63	0·067
		A>D=E=F; A=G>D=E; A=B=C=G>E; B=C=F=G; B=C=D=F								
Tops, % moisture	NS	83·74	82·48	82·80	83·46	84·18	82·91	82·43	83·14	0·583
Roots, % moisture	SS	90·54	90·60	90·42	90·35	90·30	89·39	89·52	90·17	0·284
		A=B=C>F=G; A=B=C=D=E>F; D=E=G								
Whole plants, % moisture	S	88·46	88·53	88·45	88·78	89·07	87·72	87·46	88·35	0·355
		D=E>F=G; B=D=E>G; A=B=C=D=E; A=C=F=G; A=B=C=F								

Table 2 contains the numerical data. All of the six yield values were very strongly significant at the 0·1 % point (SSS), except for the dry weights of tops, where strong significance at the 1 % point (SS) was shown. The top/root ratios and the moisture contents of the roots were strongly significant, while the moisture contents of the whole plants were significant (S) at the 5 % point, and those of the tops were uninfluenced by variations in nitrogen supply (NS).

For the fresh weights of root, A=B=C=D=E>F=G; that is, over a long range of nitrogen, 4·12–65·92 p.p.m., there was apparently no benefit to be got by increasing the nitrogen supply. An inspection of all the six sub-tables for yield, however, showed that C was in every case at least equal to A (A=C), and that except for the fresh weights of the tops, there seemed to be some bias in favour of C as the optimum concentration of nitrogen.

The data for the fresh weights of the roots were, therefore, further examined: C (11·28 g.) and D (11·12 g.) were found to be the only two yields significantly above the general mean (7·23 g.). As the general mean also contains the treatment mean under special examination, it is better to isolate the treatment means in turn: C and D, separately, were both found to be significant at the 0·1 % point against all other treatments; A was also significant at the 5 % point by this method, but B and E were not.

Objection might be taken to the above procedure on the score that the inclusion of F and G, with small treatment means, helped in this differentiation, and hence a fresh analysis of variance, comprising only the comparable treatments A–E, was made. The treatments as a whole showed no significance; but isolation of C and D grouped together against the others, A, B, and E, gave significance at the 5 % point. Correspondingly, there was no significant difference within the others; and there was no significance within C and D, also.

Thus it appears probable that the optimum range of nitrogen for the carrot in sand is 8·24–16·48 p.p.m. This is a low concentration, as may be seen by comparison with another root crop, the turnip, grown under analogous conditions with the same solutions (Woodman, 1941) (see Table 3).

The turnip may be said to respond 'normally' to nitrogen, as increased nitrogen causes a corresponding increase in yield to the limits of the experiment; it should make an ideal 'type' plant for indicating

nitrogen in soils. With the carrot, however, there is not much variation in size above 4·12 p.p.m. of nitrogen, and this crop apparently does not need heavy doses of this fertilizer. The inference is in line with practice in the eastern counties, where carrots are often grown as a catch-crop without further addition of nitrogen, and where land in good heart (e.g. in the Fens) is often not fertilized with nitrogen even for the main crop.

TABLE 3. *Comparison of the fresh weights in g. of carrot and turnip roots grown in sand culture under analogous conditions with varying nitrogen supply*

N (p.p.m.)	65·92	32·96	16·48	8·24	4·12	0·41	0·00
Carrot	9·04	8·52	11·28	11·12	8·78	1·63	0·22
Turnip	146·17	99·73	55·39	26·99	12·28	0·52	0·18

possibly the largest; F gave much smaller cultures, while G yielded tiny plants of dark bronze foliage.

The eleven values examined (Table 4) were all very strongly significant (SSS) except for top/root ratios for dry matter and the moisture contents of the whole plants, where non-significance (NS) obtained. Here, over a long range of phosphorus, there did not appear to be much variation in the yields, and actually A = B = C for all six of the yield sub-tables.

There was apparently, however, a distinct bias throughout in favour of treatment C. For the fresh roots, A, B, and C, were all significantly greater than the general mean. A new analysis of variance was drawn out for the five comparable treatments A–E, and the treatments as a whole just reached significance at the 5 % point, the new S.E. of the treatment means being 2·327. This gave the comparisons:

TABLE 4. *Summaries of results for phosphorus experiment. Weights in g.*

Description of data		Treatment mean for A	B	C	D	E	F	G	Mean of all results	S.E.
Tops, FW	SSS	6·15	5·27	6·76	4·24	3·37	0·90	0·02	3·82	0·705
		C > D = E > F = G; A = B = C > F = G; A = C > E; B = D = E; A = B = D								
Roots, FW	SSS	19·72*	20·30	22·80	13·81	15·00	4·51	0·02	13·74	1·971
		A = B = C > D > F = G; C > D = E > F = G; A = B = E								
Whole plants, FW	SSS	25·87†	25·57†	29·56	18·06	18·36	5·41	0·04	17·55	2·502
		A = B = C > D; A = C > D = E > F = G; B = E								
Tops, DW	SSS	0·97	0·82	1·05	0·71	0·58	0·17	0·002	0·61	0·119
		A = B = C = D > F = G; A = C > E > F = G; B = D = E								
Roots, DW	SSS	1·97	2·12	2·20	1·36	1·31	0·41	0·002	1·34	0·190
		A = B = C > D = E > F = G								
Whole plants, DW	SSS	2·94	2·94	3·24	2·07	1·89	0·57	0·004	1·95	0·301
		C > D = E > F = G; A = B = C > E; A = B = D > F								
Top/root, FW	SSS	0·31	0·26	0·30	0·32	0·24	0·21	1·55	0·46	0·079
		G > A = B = C = D = E = F								
Top/root, DW	NS	0·47	0·39	0·48	0·52	0·46	0·42	1·02	0·54	0·172
Tops, % moisture	SSS	84·65	84·40	84·74	83·38	82·83	81·47	88·11	84·23	0·709
		G > A = B = C = D = E; A = B = C > F; D = E = F								
Roots, % moisture	SSS	90·12	89·69	90·34	90·04	91·08	90·80	84·88	89·57	0·820
		A = B = C = D = E = F > G								
Whole plants, % moisture	NS	88·90	88·60	89·06	88·48	89·54	89·22	86·86	88·67	0·694

* A and D are just 3 × S.E. apart, and A has been put > D.
† A and E are just 3 × S.E. apart, and A has been put > E; in like case, B has been put > D.

Phosphorus experiment

The number of glazed culture jars was seventy, arranged as ten replications of a randomized block of the seven treatments. Seed sown, 18 Dec. 1940; germinated, 6 Jan. 1941; plants singled, 11 Jan.; and harvested, 18 Mar. Each culture received 28 l. of its appropriate culture solution in lots of 400 c.c. about every 2 days.

By 20 Feb., plants undergoing treatment G (0·10 p.p.m. of P) were quite small, and the cotyledons were bronzed; otherwise there was not much difference in appearance throughout the whole experiment. At harvest, 18 Mar., treatments A–E inclusive yielded good cultures with little difference in appearance and size except that those with C were

C > D = E; A = B = C; and A = B = D = E; which are somewhat similar to those in Table 4. Isolation of C *v.* A, B, D, and E, for this new analysis, gave significance at the 5 % point, with no significance within others (A, B, D, and E); this was borne out by the isolation B *v.* A, C, D, and E, where there was no significance but significance with the others (A, C, D, and E). It may therefore be assumed that C, with 6·14 p.p.m. of phosphorus, is the optimum treatment.

It is usual, at least in Fen practice, to apply extremely large amounts of phosphate for the main carrot crop, though often in some districts in East Anglia no fertilizer is applied. The present results, however, bear out those obtained for the turnip

(Woodman, 1941) where a remarkable decrease in root accompanies an increase in phosphorus (Table 5).

TABLE 5. *Comparison of the fresh weights in g. of carrot and turnip roots grown in sand culture under analogous conditions with varying phosphorus supply*

P (p.p.m.)	87·36	43·68	21·84	16·38	10·92
Carrot	—	—	—	19·72	—
Turnip	13·58	26·40	42·80	—	119·5
P (p.p.m.)	8·19	6·14	5·46	4·09	2·05
Carrot	20·30	22·80	—	13·81	15·00
Turnip	—	—	172·4	—	—
P (p.p.m.)	1·02	0·55	0·10	0·00	
Carrot	4·51	—	0·02	—	
Turnip	—	9·24	—	4·86	

Great deficiency of phosphorus caused a high top/root ratio for fresh yield, G>A=B=C=D=E=F, the presence of 1·02 p.p.m., as in F, resulting in stabilization at the lower value, as may be seen from this comparison. Similar deficiency was responsible for a high moisture content in the tops, but a low one for the roots.

Potassium and boron experiments

The number of glazed pots was seventy, arranged as ten replications of a randomized block of the seven treatments. Seed sown, 24 Aug. 1939; germination by 1 Sept.; cultures singled, 5 Sept.; and harvested, 10 Jan. 1940. Each culture received 29·6 l. of the appropriate solution in lots of 400 c.c. about every 2 days.

TABLE 6. *Summaries of results for potassium and boron experiments. Weights in g.*

Description of data		Treatment mean for A	B	C	D	E	F	G	Mean of all results	S.E.
Tops, FW	SSS	3·76	1·06	3·36	2·98	2·51	0·07	0·06	1·97	0·351
		A>E>B=F=G; A=C=D>B=F=G; C=D=E								
Roots, FW	SSS	10·72	2·07	8·78	8·48	6·51	0·06	0·03	5·24	0·963
		A>E>B=F=G; A=C=D>B=F=G; C=D=E								
Whole plants, FW	SSS	14·48	3·13	12·14	11·47	9·02	0·12	0·09	7·21	1·206
		A>E>B=F=G; A=C=D>B=F=G; C=D=E								
Tops, DW	SSS	0·51	0·15	0·51	0·41	0·37	0·01	0·01	0·28	0·053
		A=C=D=E>B=F=G								
Roots, DW	SSS	1·12	0·20	0·90	0·82	0·67	0·01	0·002	0·53	0·104
		A>E>B=F=G; A=C=D>B=F=G; C=D=E								
Whole plants, DW	SSS	1·63	0·34	1·41	1·23	1·04	0·02	0·01	0·81	0·147
		A>E>B=F=G; A=C=D>B=F=G; C=D=E								
Top/root, FW	SSS	0·37	0·75	0·42	0·40	0·41	2·06	2·91	1·05	0·395
		F=G>A=B=C=D=E								
Top/root, DW	SSS	0·49	1·14	0·64	0·56	0·56	2·60	6·31	1·76	0·572
		G>F>A=C=D=E; G>B=F; A=B=C=D=E								
Tops, % moisture	SSS	86·50	86·02	84·34	86·15	85·58	68·04	78·85	82·21	2·433
		A=D>G>F; A=B=C=D=E>F; B=C=E=G								
Roots, % moisture	S	89·73	90·24	89·72	90·14	89·73	78·97	88·27	88·11	2·539
		A=B=C=D=E=G>F								
Whole plants, % moisture	SSS	88·86	88·44	88·19	89·03	88·63	76·74	82·01	85·99	1·802
		A=B=C=D=E>F=G								

It is striking that the optimum concentration for carrots is somewhere round 6·14 p.p.m. of available phosphorus, while that for turnips is near 5·46 p.p.m. The results obtained with turnips caused the author to recast his experimental range of phosphorus for root crops more narrowly, as for the carrots in this investigation. That amounts of phosphorus prejudicial to the growth of the turnip have not a retarding effect on growth in general, is shown by the facts that 43·70 and 32·78 p.p.m. of P gave the largest yields of both fresh roots and tops with May King lettuce (Woodman, 1939), while Cheshunt Early Giant lettuce gave the largest yields of fresh roots and tops at 43·68 p.p.m. of phosphorus (Woodman, 1940). In comparing roots of plants such as lettuce with those of 'root' crops such as the turnip, it should be remembered that, with the second group, the edible portion is here included in the root together with fibre root, as in the usual horticultural definition.

Treatments A, C, D, and E yielded plants with normal tops; the roots were normal in colour, but there was a tendency to dumpiness in shape, especially with A, most potassium. Treatments F and G gave much smaller plants; there was continuous scorch and death of the oldest leaves during the experiment, with some tendency to chlorosis; and the roots were small and ill-developed, so that the proportion of fibre root appeared to be relatively large. Treatment B, lack of boron, caused chlorosis at first; but later the colour recovered, and the main effects of no boron seemed to be smallness and lack of maturity.

Except for the dry weights of the tops, where A=C=D=E>B=F=G, the six yield values, all SSS, followed the same comparison: A>E>B=F=G; A=C=D>B=F=G; C=D=E (Table 6). As regards size, therefore, lack of boron is equally as serious as lack or great deficiency of potassium

(B = F = G). A and C, for the fresh roots, were both significantly different from all the others grouped together, but in both instances there was significance within the others. A fresh analysis of variance was therefore drawn up for the comparable treatments A, C, D, and E; the treatments as a whole were found not to be significant. Isolation of A, however, proved A *v.* C, D, and E, to be significant at the 5% point with no significance within the others. C *v.* A, D, and E, was not significant, though there were significant differences within the others, A, D, and E. It thus appeared that A, with the largest concentration of potassium, was really the optimum treatment.

Deficiency in potassium tended to high top/root ratios and to lower moisture contents.

Field trials with carrots

The series of field trials that includes these experiments was described for the crop lettuce (Woodman, 1942). The present results include those for the dunged plots also. The full dressing for the NPK plots was at the rate of 1 cwt. of ammonium sulphate, 5 cwt. of superphosphate, and 3 cwt. of potassium sulphate p.a., the necessary omissions being made for the NP, NK, PK, and NIL, plots. The duplicate series of dunged plots received dung at the rate of 20 tons p.a. every 2 years. All of the nitrogen plots also received a side-dressing of nitro-chalk at the rate of 1½ cwt. p.a. at the second hoeing.

The rows were 1 ft. apart, and the carrots were thinned to 4 in. in the rows. The roots only were weighed. The variety was Primo, except for 1941, when this was unobtainable, and Ideal was substituted. The carrots were sown as a catch-crop in July following spring cabbage in the rotation, and were harvested in Sept.–Oct.

Table 7. *Yield of carrots in lb. per 1/100 acre plot. Var. Primo;* 1941, *Ideal*

	Dunged plots					Undunged plots				
Year	NPK	NP	NK	PK	NIL	NPK	NP	NK	PK	NIL
1935	—	—	—	—	—	—	—	—	—	—
1936	31·0	28·0	29·0	45·0	28·0	63·0	57·0	60·0	60·0	40·0
1937	31·6	31·8	30·9	39·3	28·9	33·3	24·7	28·1	36·8	22·6
1938	31·0	36·0	41·0	44·0	42·0	49·0	39·0	42·0	65·0	22·0
1939	—	—	—	—	—	—	—	—	—	—
1940	55·0	50·0	36·5	53·5	33·5	72·0	56·5	54·8	59·8	30·8
1941	102·8	102·0	88·1	105·8	94·1	98·6	91·9	84·9	106·9	34·6
Total	251·4	237·8	225·5	287·6	226·5	315·9	269·1	269·8	328·5	150·0
Mean	50·28	47·56	45·10	57·52	45·30	63·18	53·82	53·96	65·70	30·00

Table 8. *Comparisons between dunged plots, and between undunged plots. Means are lb. per 1/100 acre plot*

(ns = not significant; 5, 2 and 1% are the levels at which significance occurs)

	Dunged mean	NPK 50·28	NP 47·56	NK 45·10	PK 57·52	NIL 45·30	All others	All N 47·65	All P 51·79	All K 50·97	NPK + NP 48·92	NPK + NK 47·69	NPK + PK 53·90
Dunged													
NPK	50·28	—	ns	ns	ns								
NP	47·56		—	ns	ns								
NK	45·10			—	ns								
PK	57·52				—	ns							
NIL	45·30					—	ns	ns	ns	ns	ns	ns	ns
All others							—	ns	ns	ns	ns	ns	ns
Undunged	Undunged mean	63·18	53·82	53·96	65·70	30·00		56·99	60·90	60·95	58·50	58·57	64·44
NPK	63·18	—	ns	ns	ns	5%							
NP	53·82		—	ns	ns	5%							
NK	53·96			—	ns	5%							
PK	65·70				—	2%							
NIL	30·00					—	2%	2%	2%	1%	5%	2%	1%
All others							—	ns	5%	5%	ns	ns	5%

Results obtained are given in Table 7. The general mean for all plots was 51·24 lb.; the mean of the dunged plots was 49·15 lb.; and that for the undunged plots was 53·33 lb. Comparisons were made by the table of '*t*', and are given in Table 8.

In no case was there any significance between any plot, or groups of plots, in the dunged series; but it should be remembered in this connexion that a dressing of average farmyard manure of 20 tons p.a. every 2 years is equivalent to about 240 lb. of

nitrogen, hardly of any use to plants (Garner, 1934), 240 lb. of potash (K_2O), and 100–160 lb. of phosphoric acid (P_2O_5); the application of this dung, however, always fell in the autumn preceding the summer lettuce, which was followed by spring cabbage and then early carrots sown late; i.e. the dung was always applied nearly 2 years before the carrot crop.

On the undunged plots, however, the yield from any plot receiving any fertilizer, NPK, NP, NK, or PK, was significantly greater than that for the NIL plot. There were also responses for the comparisons, all others *v.* NIL, all N plots *v.* NIL, all P plots *v.* NIL, all K plots *v.* NIL, plots receiving NP *v.* NIL, those receiving NK *v.* NIL, and those receiving PK *v.* NIL. All P plots, and all K plots, but not all N plots, gave a response over all others. There seems to be some bias here in favour of P and K for the carrot as against N; and NPK+PK is the only one of three possible binary combinations of the four fertilized plots significant against all others.

A comparison of dunged and undunged plots for any identical fertilizer treatment, or of any plot or group of dunged plots with any plot or group of undunged plots, demonstrated that in only one case, PK (dunged) *v.* NIL (undunged), was there a significant response. This tended to show that over a period of 7 years' continuous cropping on this old river gravel by the 4 years' rotation of crops, brussels sprouts, peas, summer lettuce, and spring cabbage followed by the catch-crop carrots, the dunged plots plus any combination of artificials are not superior to undunged plots receiving any combination (NPK, NP, NK, or PK) of artificials. This is apparent from the data in Tables 7 and 8, which show a bias in favour of all but the NIL of the undunged plots. The table of these comparisons is not included here, as there is the one case only of significance, as detailed above.

It can be contended that, because all nitrates are soluble, the application of artificials containing nitrogen to soils is somewhat similar to feeding sand cultures by nitrate. If that be granted, then there appears to be some basis for the practice in the eastern counties of omitting nitrogen in the fertilizer applied to soils; especially if the land is in good heart, even if only by dunging, as in the NIL dunged plot, which over 7 years' cropping (five carrot harvests) has yielded equally as well as the dunged plots receiving artificials (Tables 7, 8).

The same contention cannot be made for potassium and phosphorus. It is feasible that much larger amounts of these are necessary for soils than for sand cultures, where all the 'dressings' are in solution as potassium sulphate and sodium phosphate (here), and are therefore always available, as there are no possible large-scale interactions with the sand used, which contained 99·8% of SiO_2. Bearing in mind, however, that the PK plot only, of the dunged plots, gave a response over the NIL (undunged) plot, that the PK undunged plot responded as against the NIL undunged plot, and that all undunged plots containing P, or K, but not N, gave responses over all other undunged plots, the argument may be put forward that if any dressings are applied to the carrot crop, these should consist primarily of phosphate and potash.

Influence of manuring on the incidence of carrot fly*

In 1941 the author commenced collaboration with W. H. Smith, M.A., of the D.S.I.R. Food Investigation, Ditton Laboratory, East Malling, Kent, on the storage of carrots grown under different manurial treatments. The unwashed roots from the 1941 plots were sent to Malling and, during the course of his experiments there, Mr Smith obtained the following data on the incidence of carrot fly:

Date of harvest, 21 Oct. 1941; date of storage, 25 Oct.; temperature of storage, 40° F.; the containers were trays, examined after 92 days on 22–31 Jan. 1942, and orchard boxes, examined after 151 days on 27 Feb.–4 Mar. 1942. The percentages of roots attacked by the fly are given below in descending order of magnitude; the numbers in parentheses are the actual numbers of carrots examined, while D and U indicate the dunged and undunged plots, respectively.

Trays: NPK.D (280) 74%; NK.D (291) 70%; NP.D (299) 66%; PK.D (301) 54%; NIL.D (200) 48%; NPK.U (303) 45%; NP.U (312) 32%; NK.U (297) 31%; PK.U (300) 26%; and NIL.U (—) —.

Boxes: NPK.D (123) 80%; NK.D (83) 67%; NIL.D (115) 63%; PK.D (101) 62%; NP.D (101) 61%; NPK.U (114) 46%; NP.U (106) 33%; NK.U (116) 31%; PK.U (118) 29%; and NIL.U (110) 8%.

The trend of the results of this non-replicated experiment indicates that the fly preferred the dunged plots (D) to those undunged for a number of years (U). Thus, in both the tray and box experiments, carrots from all the D plots showed greater incidence of carrot fly than those from any of the undunged plots. Carrots grown on the plots receiving full artificials (NPK) also showed greatest attack by carrot fly for both the D and U series of plots, while the NIL.U plot was scarcely affected (8%). Evidently the presence of dung (especially) and/or fertilizers predisposes the carrot to attack.

I thank Mr T. W. G. McKean and Mr J. F. N. Leonard for their great help in these experiments.

* A recent paper [Petherbridge, F. R., Wright, D. W. and Davies P. G. (1942), Investigations on the biology and control of the carrot fly (*Psila rosae* F.), *Ann. appl. Biol.* **29**, 380–92] indicates that the design of the manurial plots might have had a definite bearing on the incidence of carrot fly.

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(*Received* 1 *May* 1942)

The prevention of seed-borne diseases of flax by seed disinfection

BY A. E. MUSKETT AND J. COLHOUN

Plant Disease Division, Ministry of Agriculture, The Queen's University of Belfast

(With Plate 1)

An account is given of an investigation of the prevention of seed-borne diseases of flax by seed disinfection; the work was carried out during the 3 years 1939–41. The diseases mainly considered were stem-break and browning caused by *Polyspora Lini* Laff. and seedling blight caused by *Colletotrichum Lini* (Westerd.) Toch.

Suitably infected seed samples were selected by the Ulster method for the examination of flax seed for the presence of seed-borne parasites. A laboratory technique, based on the Ulster method, was devised for the rapid evaluation of materials likely to prove useful as seed disinfectants. This technique allows such evaluation to be made within the period of 1 week at any time during the year and the necessity for conducting extensive field trials with unpromising fungicides is thereby avoided. Only a few of the numerous substances tested in the laboratory were of sufficient promise to warrant their further investigation in the field. Organo-mercury powders of the type used for the treatment of cereal seeds were relatively ineffective unless used at an excessive dosage rate considered to be too high and wasteful for general practice; used at a normal dosage rate they gave very satisfactory results when fixed to the seed with water or separated milk (the 'fixation' method of seed treatment). Good results were obtained by the use of a suitable organo-mercurial applied by the short-wet method. A proprietary dry fungicide containing tetra-methyl thiuram disulphide as its active constituent and considered to be non-poisonous, provided almost complete control over seedling blight and some control of stem-break and browning when applied at the rate of 12 oz./cwt. of seed. Formaldehyde proved to be phytocidal when used at an effective dosage rate, while cuprous oxide was non-effective.

The prevention of attack by stem-break and browning resulted in highly significant increases in both crop and fibre yields, while in the case of seedling blight the prevention of this disease, although not reflected so much in the crop yield, produced highly significant increases in the yield of fibre. In one case at least the results suggest that effective treatment of the seed produced an increase of fibre not directly attributable to the prevention of either seedling blight or stem-break and browning; it is possible that this result may be explained by a general beneficial effect due to seed disinfection.

Results obtained by the autumn of 1940 led to the decision to disinfect all flax seed produced in Northern Ireland for the 1941 sowing. The fungicide selected was the proprietary dusting powder containing tetra-methyl thiuram disulphide (Nomersan); it was applied at the rate of 12 oz./cwt. (6·7 g./kg.) of seed. The disinfection of the seed produced throughout the country was supervised by the personnel of the Plant Disease Division of the Ministry of Agriculture. The scheme operated smoothly and allowed close supervision to be exercised over seed production generally; its adoption is being continued. In 1941 and 1942 the all-in cost of the disinfection of sufficient seed to sow one statute acre was approximately 2*s.* 6*d.*

Although Nomersan was selected for general use, this does not mean that it provides the most effective known treatment against seed-borne diseases of flax. While it provides an almost complete safeguard against seedling blight (*C. Lini*) and, in addition, serves as an acaricide and prevents the mite infestation of stored seed, it is not so effective for stem-break and browning (*P. Lini*), nor is it very satisfactory as a general disinfectant. The short-wet treatment of seed using an organo-mercurial in solution and the fixing of an organo-mercury powder to the seed by the use of water or separated milk have both been shown to be more effective methods deserving of further consideration.

The life histories of *Colletotrichum Lini* (Westerd.) Toch., the cause of seedling blight of flax, and *Polyspora Lini* Lafferty, the cause of stem-break and browning, were elucidated by Pethybridge & Lafferty (1918–22). In addition, Lafferty attempted their control by seed disinfection, but his investigations were concluded in 1922, before the work had met with more than partial success. The occurrence of occasional severe outbreaks of seedling blight and browning in the flax crop maintained interest in these diseases in Northern Ireland, and, in 1938, an attempt was made to find a simple method for the rapid and accurate examination of seed samples for the presence of *C. Lini* and *P. Lini*. A satisfactory method was found and details of the technique were published (Muskett & Malone, 1941). It at once became possible to select suitable samples for use in the study of disease prevention, and it was thought desirable to reopen the investigation commenced by Lafferty. The decision to proceed with the work was made in January 1939 when the Flax Development Committee, Northern Ireland, provided a grant-in-aid for the purpose. The investigation has been in progress for the 3 years 1939–41, and the present paper deals with the prevention of disease by seed disinfection.

Methods of evaluation of seed disinfectants

Choice of seed samples

Seed samples were selected on the basis of their being heavily contaminated with one or other of the two parasites *P. Lini* or *C. Lini* and as free as possible from any organism the presence of which might vitiate the value of the results obtained.

Laboratory technique

With slight modification, the Ulster method as used for the examination of seed samples for parasitic fungi can be used for the preliminary evaluation of seed disinfectants. Details of this modified method were published (Muskett & Colhoun, 1942), and as it has been used continuously for 3 years, ample opportunity has occurred for assessing its accuracy and reliability. A high degree of correlation exists between the results obtained by it and those from field experiments, with the result that it was found unnecessary to submit to field trials those materials which did not give promising results when tested in the laboratory. By the use of this 'sorting' test which can be made in one week at any time of the year, much of the time and labour necessary in the conduct of field trials was saved and an accurate opinion of the behaviour of any fungicide under test was rapidly obtained.

Field technique

For small-scale field trials, plots measuring 2 × 2 yd. (1·83 × 1·83 m.) or 3 × 3 yd. (2·74 × 2·74 m.) were employed. For trials on a larger scale, plots measuring 4 × 12 yd. (3·66 × 11·0 m.) or approximately 0·01 acre (40·25 m.^{2}) were used. Paths, 6 ft. (1·83 m.) in width, were left between the plots in order to minimize, as much as possible, the danger of the transmission of disease from plot to plot. The plots were randomized and grouped on the checkerboard principle, and where a statistical analysis of the results was required, they were laid down in quadruplicate or quintuplicate; in other cases a series in duplicate was arranged. The seed was sown in drills at the rate of 80 lb. (36·36 kg.) per statute acre (0·405 ha.). For each small plot the requisite quantity of seed was weighed into a paper envelope and sown by hand. The drills were spaced at 6 in. (15 cm.) intervals. After sowing, the drills were filled in and the plots lightly raked, the land having been rolled before marking them out. The large plots were sown with a small hand-operated drill.

Evaluation in the field for the prevention of stem-break and browning

The attack by *P. Lini* assumes three separate and well-defined phases which are the seedling phase, stem-break and browning. The seedling phase (Pl. 1, fig. 2) is characterized by the formation of dark-coloured circular lesions on the cotyledons. The rate of spread is very slow when compared with that of seedling blight (*C. Lini*) and the plant usually survives. The most suitable time for examining crops for the seedling phase occurs normally at 3–4 weeks after sowing, and the attack will be found to be most severe where the seedlings are thickest owing to the slight but inevitable unevenness of sowing. With the exception of lesions formed on the secondary leaves, no further signs of the disease are noticed until the plants are well grown when, about one month after the observance of the seedling phase, the symptoms of stem-break appear (Pl. 1, fig. 3). A canker is formed on the main stem at the level of the attachment of the cotyledons, and it becomes weakened. Often, it breaks completely leaving a short stump about 0·5 in. (1·25 cm.) in length protruding from the soil. In other cases it bends over sharply at the point of attack and becomes markedly 'bowed' as the result of the plant regaining its upright posture. Crop movements due to wind and rain are sufficient to cause the breaking off or intense bending of affected plants, and where the proportion of such plants is high the whole of the crop may be laid and eventually spoiled. Infection occurs through the diseased cotyledons which, after having been killed, hang down and adhere to the stem. The slow spread of the disease is again exemplified by the time which elapses between its appearance on the cotyledons and the onset of stem-break. In a normal season observations for stem-break are best made some two months after sowing. The browning phase (Pl. 1, fig. 4) appears at the end of July or in early August when the bolls are well formed and the crop is approaching maturity. It is detected by the occur-

rence of brown patches here and there in the crop; these may spread rapidly and, if the weather conditions are favourable, the whole crop may be involved within a few days of the first symptoms having been noticed. Browning is best known to the farmer who does not usually associate it with the seedling and stem-break phases which may, in cases of slight attack, go almost unobserved. It is through the browning phase that the seed becomes infected and thereby carries over the disease to the next year's crop. The lesions are most numerous in the region of the bolls and in the 'boughs'; they tend to become less frequent as the flowering branches and stem are traced downwards.

Assessment of the value of a seed treatment for preventing attack by *P. Lini* was made for each of the three phases of the disease. In each case observations were made at the most opportune time, and plots were compared by giving an assessment mark of 10 to those most severely attacked (including the controls), the marking of the others varying between 10 and 0 according to the amount of infection present. For the browning phase, weekly observations were usually made from the first appearance of the disease until the crop was pulled.

Where the fibre yields were required, the crops were pulled and handled according to the normal practice followed in the green scutching of flax. Crop weights were taken just prior to scutching and the fibre weighed immediately afterwards.

Evaluation in the field for the prevention of seedling blight

Crop attack by *C. Lini* is best observed in the seedling stages (Pl. 1, fig. 1) when the disease is characterized by typical lesions produced on the cotyledons and an orange-coloured canker on the hypocotyl at or just below soil level. The dark setae characteristic of the genus *Colletotrichum* are normally produced and the presence of the parasite readily confirmed by using a hand lens. The best time for crop examination for seedling blight is about 3 weeks after sowing.

The method used for the assessment of seed disinfectants for the prevention of seedling blight was the same as that for stem-break and browning, and a similar procedure was adopted where crops were scutched for the determination of fibre yields.

EFFECTIVENESS OF SEED DISINFECTANTS IN PREVENTING DISEASE

I. *Formaldehyde*

The use of formaldehyde for seed carrying the flax wilt organism (*Fusarium Lini*) was investigated by Bolley (1901) in the United States. Satisfactory results with no attendant crop injury were apparently obtained by sprinkling the seed with an aqueous solution of formalin (1 in 330) and drying it immediately after treatment. In Russia, Kletschetoff (1926) found a 3% solution of formalin in alcohol to which 1% naphthalene was added, to be a promising treatment for parasitic fungi, both from the point of view of eliminating seedling infection and the absence of any phytocidal effect. Flor (1936) in the United States using an aqueous solution of formalin (1 in 320) in which the seed was steeped overnight and then dried immediately afterwards, reports some injury to the stand of the crop. In his work in Ireland, Lafferty (1921) concluded that whereas the treatment of infected seed with formaldehyde solutions reduced attack by both *C. Lini* and *P. Lini*, yet the measure of control obtained was not complete and crop injury frequently resulted.

In 1939 the value of formaldehyde applied as a dilute aqueous solution of formalin* was again investigated for the prevention of stem-break and browning. Four strengths of solutions were employed ranging from 1 in 50 to 1 in 80 and each was used at two rates—20 and 40 c.c./500 g.† of seed. In each case the requisite quantity of the solution was atomized over the seed which was contained in a large Erlenmeyer flask, and the contents shaken for 5 min. The seed was then packeted and allowed to dry in the warm air of the laboratory. The 500 g. sample was sufficient for both laboratory and field tests.

At none of the strengths used did formaldehyde clear the seed satisfactorily from contamination with *P. Lini*. More promising results were obtained when the stronger solutions were applied at the heavier rate but a depression of growth was noticed. This depression persisted throughout the growing season and a poorer stand of flax frequently resulted. Because of this phytocidal effect and the failure of formalin to give more satisfactory results for disease control, its use was discontinued after 1939.‡

II. *Organo-mercurials*

The general effectiveness of fungicides containing organo-mercury derivatives as their active ingredients for the prevention of seed-borne diseases of cereal crops, suggested that they might be efficacious for flax.

Schoevers (1923) working in Holland and using solutions of proprietary fungicides of this type applied in sufficient quantity to moisten the seed which was dried immediately afterwards, reported satisfactory results for the control of *Botrytis*. He stressed the difficulty of using the wet treatment on a large scale for flax owing to the mucilaginous nature of the outer layer of the seed coat and the tendency of the seeds to cake together. None of the treatments used by van Poeteren (1926) was found to be entirely

* The sample of formalin used contained 38·0% formaldehyde.

† 20 c.c./500 g. = 0·45 gal./cwt.

‡ Detailed results are available for all experiments but, in order to save space, data from four typical experiments only are presented in this paper.

satisfactory for either *C. Lini* or *Botrytis* sp., but some degree of control was provided by the use of certain dusting powders. In Germany, Schilling (1925) investigated the use of mercury steeps and reported good results for the control of *C. Lini* and *Botrytis cinerea* as well as finding that the crop yield was increased as the result of some treatments. Work in Latvia by Eglits (1929, 1932), in which both wet and dry treatments were employed, showed that a significant but variable increase in long fibre was obtained as the result of treatment which also improved the technical qualities of the fibre and the oil content of the seed. In Russia, Zybina (1929) investigated many treatments and found some to be better than others. Burnett & Reddy (1931), using wilt-resistant varieties of linseed flax, reported from Iowa, United States, substantial increases in stand and crop yield as the result of seed treatment, while Flor (1936), also working in the United States, apparently with seed samples where the degree of contamination with seed-borne parasites was unknown, found the results of seed treatment to be so variable as to make it doubtful whether general treatment was worth while.

The incomplete nature of the work already done and the fact that it had largely been carried out with the object of investigating the general effect of seed treatment rather than its efficacy in preventing certain specific diseases, indicated the desirability of a more thorough investigation.

The dry (dusting) method. The following proprietary compounds were used; each contained an organo-mercury derivative as its active ingredient and had been found effective for the prevention of seed-borne diseases of oats. The dosages used were based on those found to be satisfactory for cereals. Available details regarding the materials used are as follows:

Product	Active ingredient(s)	% metallic mercury
Abavit B	Organically combined mercury	3·0
Agrosan G	Do.	1·5
Ceresan (1875 A)	Do.	1·5
Lunasan	Do.	0·54
TB. 910 A	Do.	Unknown

In 1939 each product was used at three dosages, 1·5, 3·0 and 6·0 g./500 g.* of seed. The powder and seed were shaken together in a large Erlenmeyer flask for 5 min. after which the seed was packeted for subsequent use. In 1940 and 1941 the dosages used were those which gave the most promising results in 1939 (Tables 1, 2).

The results from the earlier experiments indicated that the use of organo-mercury powders at dosages comparable with those effective for cereals was unsatisfactory. Similar results were recorded for all the materials used. Increasingly better results were obtained as the dosage was increased, and moderately good results were given at the rate of 6 g./500 g., but the seed was by no means completely cleared of the parasites. These observations are true in the main for both *P. Lini* and *C. Lini*, although *P. Lini* has consistently proved to be the more difficult parasite to eradicate. Fairly satisfactory results would undoubtedly have ensued from the treatment of seed with organo-mercurial powders at this excessive dosage, but it was never recommended in practice owing to the possible danger involved by the use of such a large quantity of a poisonous dressing and the wastage of material which would result.

The short-wet method. As an alternative to the dry treatment, this method has been employed successfully on a farm scale in Northern Ireland for the disinfection of seed oats. It consists of dosing the seed with the fungicide in liquid form, the quantity of solution being small enough to make a special drying operation unnecessary. The seed is bagged as it is treated and dries naturally in the bag. For seed oats a 3·5 % solution of Ceresan (U. 564) is used and is applied at the rate of 2 l./50 kg.* Ceresan (U. 564) was used in this investigation; it contains 3·5 % of metallic mercury which is organically combined. Three concentrations of the solution were used, viz. 2, 4 and 8 %, and these were applied to the seed at the three rates of 20, 30 and 40 c.c./500 g.* The treatment of small quantities of seed was carried out by shaking the seed and solution together in a large Erlenmeyer flask for 5 min. The seed was then packeted and stored in the laboratory. No difficulty was experienced in the treatment of flax seed by this method. The moisture content of the seed samples used varied between 6·0 and 9·0 %, and in all cases the seed dried out quickly and satisfactorily (Tables 1, 2).

For the treatment of flax seed the higher application rates were found necessary. The 20 c.c./500 g. rate was too low and the best results were obtained when 30 or 40 c.c. were used. The short-wet method of seed treatment proved to be superior to the dry, and very promising results were given by the use of an 8·0 % solution at the 30 or 40 c.c. rate. The next best results were obtained by using a 4 % solution at the 40 c.c. rate.

The fixation method. To determine whether the performance of organo-mercury powders could be improved, an attempt was made to fix the dust to the seed by the use of water or separated milk. The results from preliminary experiments suggested that this method warranted further investigation, and in 1941 the fixation method of treatment was included in the programme of larger field trials. Ceresan (1875 A) was the fungicide selected. In the first place the seed was treated with the powder by the usual method at the rate of 3·0 g./500 g. This rate

* 1·5 g./500 g. = 5·33 oz./cwt. of seed.

* 20 c.c./500 g. = 2 l./50 kg. = 0·45 gal./cwt.

TABLE 1. *The prevention of stem-break and browning* (P. Lini) *and seedling blight* (C. Lini) *by seed disinfection*, 1940

Seed samples used—Blue Star (1939) for *P. Lini*, Liral Crown (1939) for *C. Lini*.
Field trials carried out at the Agricultural Research Institute, Hillsborough, Co. Down.
Plots (in quintuplicate), 3 × 3 yd. Date of sowing 13–18 May 1940. Date of pulling 22–24 Aug. 1940.
Field observations recorded as the calculated mean for each series of plots receiving similar treatments.

		Control of stem-break and browning					Control of seedling blight	
			Field test. Maximum attack = 10					
					Browning			
Treatment	Rate of application/500 g.	Laboratory test. (500 seeds examined) % infected seeds	Seedling phase	Stem-break	1st observation (1. viii. 40)	Mean of all observations	Laboratory test. (500 seeds examined) % infected seeds	Field test. Maximum attack = 10 Seedling phase
Untreated	—	23·7	10	10	10	10	27·0	10
Abavit B	3·0 g.	19·8	6	7	6	6	15·8	3
,,	6·0 g.	13·6	6	4	4	4	12·0	2
Agrosan G	3·0 g.	15·4	5	5	5	5	16·4	3
,,	6·0 g.	6·8	3	4	4	3	9·8	1
Ceresan (1875A)	3·0 g.	22·0	6	6	8	7	17·8	3
,,	6·0 g.	12·6	4	9	5	5	15·0	1
Lunasan	6·0 g.	17·2	5	5	4	4	11·2	4
TB. 910A	6·0 g.	12·4	5	6	6	5	14·2	1
Nomersan (RD. 7846)	1·5 g.	13·2	5	9	4	4	1·2	2
,,	3·0 g.	11·8	4	5	4	4	0·4	2
,,	6·0 g.	5·0	1	0	4	4	0·0	0
Ceresan (U. 564) 2%	40 c.c.	20·2	4	6	3	4	18·4	3
,, 4%	20 c.c.	19·8	5	8	6	6	19·4	3
,, 4%	40 c.c.	11·2	2	2	4	3	14·8	0
,, 8%	20 c.c.	16·0	4	7	4	4	16·2	2
,, 8%	40 c.c.	6·8	1	0	2	2	5·0	0

TABLE 2. *The prevention of stem-break and browning* (P. Lini) *and seedling blight* (C. Lini) *by seed disinfection*, 1941

Seed samples used—Stormont Gossamer (1940) for *P. Lini*, Liral Monarch (1940) for *C. Lini*.
Field trials carried out at the Agricultural Research Institute, Hillsborough, Co. Down.
Plots (in quadruplicate), 0·01 acre. Date of sowing 7–14 May 1941. Date of pulling 26 Aug.–2 Sept. 1941.
Field observations recorded as the calculated mean for each series of plots receiving similar treatments.

		Control of stem-break and browning				Control of seedling blight	
			Field test. Maximum attack = 10				
Treatment	Rate of application/500 g.	Laboratory test. (500 seeds examined) % infected seeds	Seedling phase	Stem-break	Browning* 1st observation 14. viii. 41	Laboratory test. (500 seeds examined) % infected seeds	Field test. Maximum attack = 10 Seedling phase
Untreated	—	50·4	10	10	10	14·6	10
Agrosan G	6·0 g.	37·8	7	5	6	2·6	0
Ceresan (1875A)	6·0 g.	23·6	7	7	7	5·6	0
Ceresan (1875A) fixed with separated milk	3·0 g. + 40 c.c.	15·8	3	2	4	2·4	0
Nomersan (RD. 7846)	1·5 g.	35·6	7	7	8	0·8	0
,,	3·35 g.	28·2	7	7	7	0·0	0
,,	6·0 g.	19·6	6	6	7	0·6	0
Nomersan (RD. 7846) fixed with separated milk	3·0 g. + 40 c.c.	27·6	6	5	8	0·4	0
Ceresan (U. 564) 4%	40 c.c.	31·4	6	5	7	4·6	0
,, 8%	30 c.c.	29·4	5	4	7	4·2	0
,, 8%	40 c.c.	20·4	3	2	4	0·6	0

* Owing to the rapid spread of browning in 1941 only one detailed observation was possible.

was chosen as one which, judging from experience with seed oats, allowed for the greater surface area of flax seed* and provided an adequate cover. Afterwards the seed was submitted to the short-wet treatment with water or separated milk at the 40 c.c./500 g. rate. No difficulty was experienced, and it was observed that the powder was satisfactorily fixed to the seed (Table 2).

The efficacy of the organo-mercury powder was increased by its fixation to the seed. The results showed a decided improvement upon those obtained by the use of the dry treatment alone at twice the dosage, and were comparable with those given by the short-wet treatment using an 8% solution of an organo-mercurial applied to the 40 c.c. rate. Although it was only possible to use one material for these experiments there is no reason why the efficacy of organo-mercury powders in general should not be increased by their fixation to the seed. Both water and separated milk served equally well as fixation agents.

III. *Cuprous oxide*

Copper as cuprous oxide has shown promise in some instances as a seed disinfectant, and its efficacy in the case of flax was investigated. The material used had been prepared according to the specification of Horsfall *et al.* (1934), and the powder was applied to the seed at the rate of 1·5 and 3·0 g./500 g. The material proved to be ineffective and was not used after 1939.

IV. *Tetra-methyl thiuram disulphide*

The dry (dusting) method. Among the numerous samples received for test purposes from the chemical industry was a product from Messrs Imperial Chemical Industries, Ltd., coded as RD. 7846 and stated to contain tetra-methyl thiuram disulphide as its active constituent. It was in the form of a white powder and regarded as non-poisonous.† It was used in 1940 and 1941 at the rates of 1·5, 3·0, 3·35 and 6·0 g./500 g. (Tables 1, 2). The promising nature of RD. 7846 (later named Nomersan) was noted after a series of preliminary laboratory trials had been completed and was confirmed by the results obtained from field trials. Its action is selective, for whereas when applied at the 3·0 g. rate it almost eradicates *C. Lini* from infected seed, its effect on *P. Lini* is not very satisfactory and is less than that of organo-mercury powders used at the 6·0 g. rate. This same selectiveness was noted in its suppression of the growth of moulds contaminating the seed; its bactericidal qualities were poor.

The fixation method. In 1941 an experiment was included to determine whether Nomersan would give better results if fixed to the seed with water or separated milk. The same method was employed as with organo-mercury powders and Nomersan was used at the 3·0 g. rate (Table 2). No advantage was found to accrue from the fixation of Nomersan to the seed.

V. *Miscellaneous*

Numerous likely substances were tested either by the laboratory method alone or by both laboratory and field trials. They included mercuric chloride, ethyl alcohol, ether, carbon tetrachloride, trichlorethane (Fischer & Scharrer, 1925), sulphuric acid, hydrochloric acid, boric acid, chromic acid, carbolic acid, an alcoholic solution of iodine, malachite green, copper sulphate, Burgundy mixture, potassium dichromate, potassium permanganate and liver of sulphur, as well as a large number of proprietary materials submitted for test purposes by the chemical industry. None was found to be very satisfactory, and with few exceptions the trials made with them were not carried further than the preliminary stages. One interesting observation was made. When carrying out the laboratory test with seed treated with ineffective materials by the short-wet method, it was noticed that the percentage of contaminated seeds was frequently much greater after treatment than before; this was found to be due to the carrying of spores by the liquid from infected to non-infected seeds and the failure of the treatment to destroy them.

Effect of seed disinfection upon crop and fibre yields

The crops from plots laid down in 1940 and 1941 were pulled and stacked for the purpose of determining the crop weights and fibre yields. The produce from each plot was kept separate and green scutched, the crop weight being determined immediately before scutching and the fibre weight immediately afterwards. In 1940 the plots were small (3 × 3 yd.) and the crop was not de-seeded before scutching, but in 1941, when the plots were considerably larger (0·01 acre), de-seeding was carried out prior to scutching. The results for 1941 are presented in Table 3.

Analysis of variance (Fisher & Yates, 1938) was applied to the data for crop weights and fibre yields obtained for each experiment. In the case of the

* Assuming the surface area of a quantity of seed oats to be 1, then that of a similar quantity of flax seed is approx. 2·25. The normal treatment of seed oats at the rate of 5·33 oz./cwt. would therefore be equivalent to a treatment rate of 12·0 oz./cwt. (3·35 g./500 g.) for flax.

† Flax seed heavily dosed with RD. 7846 was fed to calves and pigeons over a period of many weeks and in neither case was any ill effect observed. The calves fed with the treated seed thrived equally well as the control group which received similar rations of untreated seed. The pigeons were inclined to refuse the treated seed in the first instance, but after a time they took it without hesitation. These trials are not regarded as conclusive but they indicate that the material is comparatively harmless and non-poisonous to stock.

two experiments concerned with the prevention of stem-break and browning, the following values of z calculated for treatment were obtained:

1940: crop weight + 0·42131, fibre yield + 0·32054 [5 % point 0·30 ($n_1 = 16$, $n_2 = 64$)]

1941: crop weight + 1·62035, fibre yield + 1·07263 [0·1 % point 0·72 ($n_1 = 10$, $n_2 = 30$)]

In each case treatment produced a real effect upon crop weight and fibre yield. Using the standard deviation for error obtained in each analysis of variance, the standard error was calculated for the differences between pairs of mean values in the same experiment. All the treatments employed in 1940 (see Table 1), except TB. 910A at the rate of 6·0 g./500 g. of seed and Ceresan (U. 564) 4 % at the rate of 20 c.c./500 g. of seed, significantly increased the crop yield over that of the controls. In 1941 all the treatments employed (see Table 3) produced a significant increase in the crop weight. Similarly, the standard error for the differences between pairs of mean values for fibre yield was calculated, and it was found that in 1940 the following treatments (per 500 g. of seed) significantly increased the yield: Agrosan G 6·0 g., Ceresan (1875 A) 3·0 and 6·0 g., Lunasan 6·0 g., Nomersan (RD. 7846) 1·5, 3·0 and 6·0 g., and Ceresan (U. 564) 8 % at 40 c.c. The treatments which significantly increased the fibre yield in 1941 are shown in Table 3.

Analysis of variance was also applied to the data for crop weights and fibre yields obtained in each of the experiments dealing with the prevention of seedling blight. The following values of z for treatment were obtained:

1940: crop weight + 0·22351, fibre yield + 0·35166 [5 % point 0·30 ($n_1 = 16$, $n_2 = 64$)]

1941: crop weight + 0·37009, fibre yield + 0·68507 [1 % point 0·549; 5 % point 0·389 ($n_1 = 10$, $n_2 = 30$)]

In the case of crop weight, treatment did not produce a real effect in 1940 or 1941, since in neither case did the calculated value of z exceed the appropriate 5 % point. In the case of fibre yield, treatment produced a real effect in both years. As in the experiments dealing with browning, the standard error for differences between pairs of mean values for fibre yield in each experiment was calculated. The following treatments (per 500 g. of seed) significantly increased the yield in 1940: Abavit B 6·0 g., Ceresan (1875 A) 3·0 and 6·0 g., Lunasan 6·0 g., Nomersan (RD. 7846) 1·5, 3·0 and 6·0 g., and Ceresan (U. 564) 4 % at 20 c.c. and 8 % at 20 c.c. The treatments which brought about significant increases in 1941 are shown in Table 3.

In the case of stem-break and browning the application of almost all seed treatments significantly increased the crop weight above that of the controls;

TABLE 3. *The prevention of stem-break and browning* (P. Lini) *and seedling blight* (C. Lini) *by seed disinfection, 1941. Crop and fibre yields*

Seed samples used—Stormont Gossamer (1940) for *P. Lini*, Liral Monarch (1940) for *C. Lini*.
Field trials carried out at the Agricultural Research Institute, Hillsborough, Co. Down.
Plots (in quadruplicate), 0·01 acre. Date of sowing 7–14 May 1941. Date of pulling 26 Aug.–2 Sept. 1941.
Results recorded as the calculated mean for each series of plots receiving similar treatment.

		Control of stem-break and browning				Control of seedling blight			
		Crop yields		Fibre yields		Crop yields		Fibre yields	
Treatment	Rate of application/500 g.	Per plot lb.	Per acre cwt. (calc.)	Per plot lb.	Per acre cwt. (calc.)	Per plot lb.	Per acre cwt. (calc.)	Per plot lb.	Per acre cwt. (calc.)
Untreated	—	18·45	16·5	0·46	0·41	64·94	58·0	5·20	4·64
Agrosan G	6·0 g.	**35·86**	32·0	**1·24**	1·11	75·16	67·1	**7·62**	6·80
Ceresan (1875 A)	6·0 g.	**35·31**	31·5	**1·12**	1·00	76·64	68·4	**6·78**	6·05
Ceresan (1875 A) fixed with separated milk	3·0 g. / 40 c.c.	**53·34**	47·6	**2·14**	1·91	78·89	70·4	**6·66**	5·95
Nomersan (RD. 7846)	1·5 g.	**29·89**	26·7	0·76	0·68	72·58	64·8	5·81	5·19
,,	3·35 g.	**29·84**	26·6	0·83	0·75	69·84	62·4	5·79	5·18
,,	6·0 g.	**35·44**	31·6	**1·24**	1·11	78·00	69·6	**6·75**	6·03
Nomersan (RD. 7846) fixed with separated milk	3·0 g. / 40 c.c.	**36·14**	32·3	**1·02**	0·91	71·16	63·5	5·92	5·29
Ceresan (U. 564) 4 %	40 c.c.	**43·17**	38·5	**1·26**	1·13	77·56	69·2	**6·56**	5·86
,, 8 %	30 c.c.	**42·66**	38·1	**1·34**	1·20	76·61	68·4	**6·87**	6·14
,, 8 %	40 c.c.	**52·30**	46·7	**1·98**	1·76	74·08	66·1	6·02	5·38

Mean values for treatments showing significantly greater yields than that for the control are given in heavy type.

in 1940 the best treatments resulted in the production of crops of over 2·5 tons/acre as against 1·65 tons given by untreated seed, while in 1941 the corresponding results were 2·0 and 0·8 tons respectively. Only the most promising treatments resulted in a significant increase in the yield of fibre; in 1940 the best treatments yielded some 3·75 cwt./acre as against the 2·5 cwt. obtained from the controls, while in 1941 the corresponding yields were 1·76–1·91 and 0·41 cwt. The reason why a significant increase in the fibre yield was not obtained whenever the crop yield was increased may be explained by the fact that in those cases where the treatment was but partially effective and allowed the comparatively rapid spread of browning, a proportion of the fibre was injured and reduced to tow during scutching.

The case of seedling blight is different. Here, in neither season was a significant increase in the crop weight recorded as the result of seed treatment, but in both seasons a significant increase in the yield of fibre was recorded for the best treatments. In both 1940 and 1941 these produced some 6·25 cwt. of fibre/acre as against 5·0 cwt. from the controls. It is suggested that the higher fibre yields may be accounted for by the production of a more even crop of healthy plants from the disinfected seed.

Seed disinfection in practice

With the outbreak of war in the autumn of 1939 and the necessity for obtaining the fullest possible crop, the results for that year were examined to ascertain whether they could be made use of with advantage in 1940. Seed treatment with an organo-mercurial offered the best line of approach. The short-wet method was of distinct promise, and the dry treatment had given fair results when the powder had been applied at the rate of approximately 21 oz./cwt. of seed. Too little was known concerning the short-wet method to allow of its wide adoption, and the dressing of the seed with a poisonous powder at the 21 oz. rate was regarded as too heavy for general recommendation. It was therefore decided to carry out the large-scale treatment of seed with an organo-mercury powder at the rate of 10 oz./cwt., more with the object of gaining experience than with the hope of obtaining outstanding results. Co-operation had already been established with Messrs R. Stevenson and Son, Ltd., Moygashel, Dungannon, and all seed produced at Moygashel in 1939–40 and contaminated to the extent of more than 5 % with either *P. Lini* or *C. Lini* or both, was disinfected. In all some 25 tons were treated. No difficulties were experienced, nor were any complaints received concerning crop damage resulting from seed treatment. Any reports received suggested that the treatment had been beneficial.

By the autumn of 1940 more knowledge had been gained. Results from seed treatment with organo-mercurials confirmed those obtained in 1939 and, in addition, tetra-methyl thiuram disulphide (Nomersan) had been submitted to thorough trial in the field. The short-wet method using an organo-mercurial in solution still proved to be very satisfactory. Nomersan had given excellent results in so far as the prevention of seedling blight was concerned, although it had not proved to be so effective for stem-break and browning; it also had the advantage of being non-poisonous. Some heavy infections with *C. Lini* were recorded in the 1940 seed crop, and it was decided that all seed produced in Northern Ireland should be disinfected with Nomersan at the rate of 12 oz./cwt. The Strickland's Dry Seed Dresser had proved to be particularly suitable for disinfecting cereal seeds in bulk, and after extensive trials it was found suitable and selected for use with flax. One or more machines were installed at each of the six main centres of seed production, and the supervision of the work was carried out by the personnel of the Plant Disease Division. To ensure that no unsuitable seed was disinfected, special care was taken with the testing of the seed before treatment for its germination capacity, percentage purity and moisture content. Some 1465 tons of high-grade sowing flax seed were produced in Northern Ireland in 1940–1 and 1429 tons of this were disinfected; the remainder was produced too late for sowing. In addition, 669 tons of incoming seed from Great Britain were treated so that, in all, 2098 tons were disinfected for the 1941 sowing.

Flax seed is usually sown broadcast in Northern Ireland using a 'fiddle' for the purpose, and the only complaints received concerned the irritation of the eyes of the sower by the free powder blowing from the seed during sowing, and cases of slight skin irritation when the treated seed was handled without due care. In either case the irritation is of comparatively short duration and can be avoided by taking suitable precautions.

Opportunity was taken in 1941 for testing the short-wet method of seed disinfection on a semi-large scale using a solution of the organo-mercurial-Ceresan (U. 564). Co-operation with Messrs R. Stevenson and Son, Ltd., was again arranged, and in all some 30 tons of seed were treated at that centre by this method. The fungicide was used at a concentration of 8 % and at the rate of 0·657 gal./cwt. (30 c.c./500 g.). Treatment was carried out using a Kontramix machine. Equipment of this type is used in Northern Ireland for the short-wet treatment of cereal seeds on a farm scale, and it proved equally suitable for flax. Before treatment, the seed had a moisture content varying from 7·0 to 9·0 %, and although it was wet and caked together after treatment, yet it dried well in the bag in the course of a few hours and no difficulty was experienced when sowing. No complaints were received regarding this seed and, on the whole, the first trial of this treatment on a large scale may be regarded as satisfactory.

Nomersan was adopted for general use with flax seed in the United Kingdom in 1941, and it was arranged that all seed used for the 1942 sowing should be dressed. Some sixteen seed-producing centres were operating in Northern Ireland, and the same system of seed certification and disinfection was employed as in 1941.

Cost of seed disinfection

In the case of Nomersan which was supplied at the bulk rate of 2*s.* 10*d.* per lb., the cost of sufficient material (12 oz.) to disinfect 1 cwt. of seed = 2*s.* 1½*d.* Taking 84 lb. of seed as sufficient to sow one statute acre, then

	s.	*d.*
Cost of sufficient material (9 oz.) to disinfect the seed required for one statute acre =	1	7·125

Assuming a charge of 9*d.* per bag to cover adequately the cost of labour involved for disinfection, including re-bagging of the seed should this be necessary, porterage and other incidentals, then

Cost of labour involved in the disinfection of the seed required for one statute acre =		6·75

Depreciation on equipment assuming cost of machinery and installation to be £100, its life to be 5 years and 150 tons of seed to be dealt with annually, then

Depreciation charge on the seed required for one statute acre =		1·2
∴ *Total cost for disinfection of seed to sow one statute acre* =	2	3·075

The maximum cost per acre for seed disinfection using Nomersan as the fungicide can therefore be assessed at 2*s.* 6*d.*

Discussion

Seed disinfection assists materially towards clearing flax seed from contamination with seed-borne parasites, thereby preventing the diseases which they cause. In the case of seedling blight (*C. Lini*), treatment with organo-mercury powders substantially reduced infection but only when used at an excessive dosage rate. Used at a rate comparable with that found to be effective for cereals, they were not very satisfactory. That their non-effectiveness is in some measure due to their failure to adhere to the seed is suggested by the finding that better results are obtained when they are applied and afterwards fixed to the seed by the use of water or separated milk. The fixing of the powder to the seed has the further advantage of eliminating the presence of free powder and thereby obviating the unpleasantness caused during sowing by the presence of excess dust. The short-wet method, using an organo-mercurial in solution, proved to be satisfactory, but the problem was further simplified when it was found that tetra-methyl thiuram disulphide as RD. 7846 (Nomersan) gave highly satisfactory results; no advantage accrued from fixing Nomersan to the seed.

The prevention of stem-break and browning (*P. Lini*) proved to be more difficult. No suitable treatment was found which will eliminate this parasite entirely from heavily contaminated seed samples, although very satisfactory results were given by the short-wet method and also by the fixation of an organo-mercury powder to the seed. Organo-mercury powders used alone showed increasing effectiveness with increasing dosage, but the effective rate was considered too high for practical purposes. Tetra-methyl thiuram disulphide as Nomersan is not so effective for the prevention of this disease, its value being below that of organo-mercury powders, and no improvement was obtained by fixing it to the seed. The selective action of tetra-methyl thiuram disulphide is of interest, for whereas it serves as an almost perfect fungicide for *C. Lini*, it is only partially effective for *P. Lini*, and its action in the suppression of moulds and bacteria generally is not very effective. Martin (1940) refers to the thiuram sulphides as being weakly fungicidal and it is possible that the effectiveness of this material for *C. Lini* is due to the intolerance of the fungus for sulphur, a point which has not been investigated. In considering the prevention of stem-break and browning due attention must be given to the importance of each phase of the disease. The outbreak of the seedling and stem-break phases may be largely prevented by suitable seed treatment and the crop thereby greatly improved, but if a trace of the disease is present it will suffice to produce an adequate inoculum to start an attack of browning when the crop is reaching maturity. The extent of its spread will then depend upon the weather conditions prevailing at the time. An epidemic of browning may occur therefore in crops grown from disinfected seed. It has been observed in all three seasons, however, that this phase of the disease spreads more slowly in crops grown from seed disinfected with an effective fungicide. This time lag prolongs the period by some 10–14 days during which the crop may be pulled before the disease has become widespread, and it may be of the greatest importance in allowing the crop to be saved in good condition. The fact that seed disinfection does not exert complete control over *P. Lini* and, under certain conditions, only results in slowing down the attack of browning, indicates that it must be adopted as routine practice each season with the object of keeping the infection of seed stocks as low as possible. An almost parallel example occurs in the case of *Helminthosporium* disease of oats (Muskett, 1937), where seed disinfection prevents almost completely the primary or

leaf-stripe phase of the disease, but where the secondary or leaf-spot phase, arising from a trace of the primary phase, may become epidemic when the crop is approaching maturity and cause heavy infection of the seed. An important difference, however, is that whereas the secondary phase of *Helminthosporium* disease is comparatively harmless and is chiefly detrimental in causing seed infection, the browning phase of *P. Lini* not only gives rise to seed infection but causes severe damage to the flax.

Any economic consideration of the benefits of seed disinfection must take into account whether or not the fibre yield of the crop is increased or improved thereby, for if the diseases caused by seed-borne parasites do not reduce the quantity or quality of the fibre, seed disinfection could be regarded as unnecessary. That substantial increase in the fibre yield is obtained as the result of the suppression of disease is shown by the results from the trials carried out in 1940 and 1941 where the crops were pulled and scutched and the crop weights and fibre yields determined.

In the case of stem-break and browning, a significant increase in the crop weight was obtained in both seasons, from nearly all the seed treatments employed. Significant increases in the fibre yield occurred with less frequency and generally resulted from the use of organo-mercury powders and Nomersan at the excessive rate of 21 oz./cwt., the short-wet method using an 8 % solution of an organo-mercurial at 0·67 or 0·9 gal./cwt., and an organo-mercury powder used at the 12 oz. rate and fixed to the seed. In other words, whereas most treatments resulted in an improvement in the crop yield, only the most promising produced a substantial increase in the yield of fibre. This may be explained by the fibre having been damaged by browning and also by the weathering of lodged crops attacked by stem-break. In such cases, although the crop weights may not vary substantially, a decrease in the yield of long fibre occurs owing to the injured and denatured fibre being reduced to tow during scutching. Only those treatments therefore which will almost eliminate stem-break and delay an attack of browning for as long as possible can be regarded as satisfactory in securing substantially increased fibre yields. The results from the 1941 trials are of special interest. Throughout the season the weather conditions aggravated attack by stem-break and browning and operated against the healthy growth and development of the crop. 50 % of the seed was infected with the parasite; this is abnormally high, and similar samples have been rarely encountered. The attack of stem-break was extremely severe, and satisfactory prevention only resulted where the seed had been treated by the short-wet method or by an organo-mercury dust fixed to the seed. By mid-July severe lodging had occurred in most of the plots, and at pulling time the control crops were completely laid, and only those grown from seed submitted to these two treatments were standing. Browning appeared suddenly in early August and spread so rapidly that it was only found possible to make one series of observations on the severity of the attack. Pulling had to be delayed for a fortnight on account of adverse weather conditions. The yields were low, but, nevertheless, the best treatments raised the crop yield from under 1 ton/acre to considerably more than 2 tons, while the fibre yield, although very disappointing, was increased from 0·41 to 1·76–1·91 cwt. The low fibre yield from the controls resulted from the weathering of the laid crop (due to stem-break) and the heavy attack of browning, while those from the treated plots were lowered by the browning attack and would undoubtedly have been much higher if the crops could have been pulled before it had become so severe.

Unless it cannot be avoided, seed stocks so heavily infected with *P. Lini* should not be used for sowing; they have been encountered but rarely in practice and should be used as feeding stuffs or else destroyed. All seed produced in Northern Ireland during the past 3 years has been examined by the Ulster method for the presence of seed-borne parasites, and this practice readily admits of the selection and rejection of such stocks.

Earlier sowing of the crop tends to advance the time of pulling, and this assists towards the prevention of damage caused by browning. The time gained through earlier pulling, coupled with the lag in the spread of the disease resultant upon seed treatment, should help materially in allowing the crop to be safely harvested before it becomes seriously affected. The damage supposed to be caused to early sown crops by frost is exaggerated; in part it is probably due to the crop being attacked by disease in the seedling stage, and this danger can be largely overcome by the use of disinfected seed.

For seed infected with *C. Lini*, no significant increases in the crop weight were obtained as the result of treatment, but significant increases in the fibre yield were regularly obtained from the best treatments. The probable explanation is that although a proportion of the seedlings in the controls was killed by disease, sufficient was left to produce a bulk of crop comparable with that obtained from treated seed. At the same time, a certain crop irregularity would result due to (1) some plants having become attacked but not killed and thereby producing weakly specimens, and (2) others, which although healthy would produce plants with coarse, woody stems, through being too widely spaced. The production of plants of either type would lead to a reduced fibre yield. On the other hand, the production of a regular crop of healthy, evenly spaced plants by seed which had been effectively treated would result in an increased yield of fibre.

Little or no reference has been made to seed-borne parasites other than *P. Lini* and *C. Lini*, although other fungi such as *Botrytis* sp., *Phoma* sp., and

Fusarium Lini, etc., which can cause disease in the crop are known to be seed-borne, and there are, in addition, numerous moulds and bacteria which occur as seed contaminants. The seed samples chosen for this work were selected because of their heavy contamination with the parasites under special investigation, but they were certainly not free from general contamination with moulds and bacteria although they were observed to be free from more than a trace of infection with other known parasites capable of detection by the use of the Ulster method. It will therefore be of interest to see if there is an indication of any general advantage accruing to seed treatment apart from the prevention of seedling blight, stem-break and browning. In seed oats, for example, where disinfection is carried out chiefly to prevent smuts (*Ustilago* spp.) and leaf-stripe (*Helminthosporium Avenae*), an additional benefit can often be traced which is not attributable to such action. For the seed samples used in both seasons for the control of *P. Lini* and for that used in 1940 for the control of *C. Lini* there is nothing which would seem to suggest any benefit other than that obtained by the prevention of the diseases under investigation, but for that used for the control of *C. Lini* in 1941, a point of interest does arise. This sample contained only 14·6% of seeds infected with *C. Lini*, and as seedling blight could not be found in any of the plots sown with seed submitted to any of the treatments employed, it is assumed that, for this season, all gave complete control of the disease. Nevertheless, significant increases in the fibre yield were not obtained in all cases, and in particular when Nomersan was used at the two lower dosages. If the total benefit due to seed disinfection in this case was attributable to the prevention of seedling blight, then a significant increase in the fibre yield should also have been obtained in these two instances. Reference has already been made to the fact that except for the control of *C. Lini*, the other treatments were much superior to Nomersan as general disinfectants, and it may be that in the case of flax seed, as in oats, there are instances when disinfection carries with it a benefit other than that which can be attributed to the control of specific seed-borne parasites, the life histories of which are well known. The non-significant increase in fibre yield obtained by the use of Ceresan U. 564 at 8% and 0·9 gal./cwt. in this particular case is attributed as likely to be due to slight phytocidal action and not to the ineffectiveness of the fungicide.

This investigation has been concerned not only with fundamental research commenced in the laboratory and carried to the field, but has embraced the application of the results to industry and has concluded only when that application has been made complete by the disinfection of all flax seed produced in the country. The choice of a fungicide containing tetra-methyl thiuram disulphide as its active constituent after less extensive trial than might have been considered adequate in more normal times, has been found to be more than justified as the result of further experience, and it is of some interest to note that the dressing also serves as a suitable acaricide. The infestation of flax seed by mites after it has been stored for some time is a common occurrence, particularly if its moisture content is high; in no case has treated seed stored over winter been found to be so infested, whereas the infestation of untreated seed stored alongside has been found to be fairly general. The relatively non-poisonous nature of the disinfectant and its harmlessness to seed and crop are also points in its favour, but at the same time it cannot be claimed as the most effective treatment, and it is not unlikely that after further experimental work has been carried out, even more effective methods for the treatments of flax seed will be found.

The disinfection of sufficient seed to sow 1 acre cost approximately 2*s*. 6*d*. in 1941 and 1942, and it is unnecessary to stress further the low premium involved in insuring the flax crop against a large proportion of the damage which might occur from the onset of attack by seed-borne diseases.

Acknowledgement is made to the Flax Development Committee for its generosity in providing a grant-in-aid in support of this work. To their colleagues and especially to Mr E. L. Calvert, B.Agr., the writers proffer their best thanks for assistance given at all times; the help given by Messrs J. P. Malone and W. Wilson has been much appreciated. Field facilities provided and the loan of equipment from time to time by the Agricultural Research Institute, Hillsborough, and the Plant Breeding Division, Stormont, are gratefully acknowledged, as also is the help given by the Seed Testing Station in dealing with the large numbers of seed samples submitted for testing. To Messrs R. Stevenson and Son, Ltd., Moygashel, Dungannon, the writers express gratitude for arrangements made in providing facilities for the scutching of the crops. The assistance given by the Chemical Industry—Messsr Bayer Products, Ltd., Messrs Imperial Chemical Industries, Ltd., and Messrs British Schering, Ltd., in submitting samples of likely seed disinfectants has been of the greatest value, while Messrs Plant Protection, Ltd., in making available adequate supplies of the selected fungicide, assisted in allowing the work to be carried through from the experimental stages to its general application in the shortest possible time.

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EXPLANATION OF PLATE 1

Fig. 1. Two flax seedlings affected with seedling blight (*C. Lini*). Both cotyledons of the seedling (*a*) have contracted the disease, while in the case of (*b*) only one cotyledon is affected. Both seedlings have contracted infection in the region of the hypocotyl. It is unlikely that either would have survived the attack (slightly enlarged).

Fig. 2. Two flax seedlings affected with the seedling phase of attack by *P. Lini*. In each case both cotyledons have contracted the disease which produces rather typical circular lesions on the leaf and spreads much more slowly than seedling blight. The stems remain unaffected. In each case the seed coat is still attached to the seedling.

Fig. 3. Mature flax plants affected with the stem-break phase of attack by *P. Lini*. The 'bowed' appearance of the plants and their restricted development are typical. ($\times \frac{1}{3}$.)

Fig. 4. Flax stems affected with the browning phase of attack by *P. Lini*. ($\times 2$.)

Fig. 5. Experimental plots at the Agricultural Research Institute, Hillsborough, in 1940, dealing with the prevention of attack by *P. Lini* by seed disinfection. In the foreground is one of the control plots sown with seed, a fairly high percentage of which was infected with the parasite; the lodging of the crop is due to the attack of stem-break. The plot in the immediate background to the left was sown with seed from the same sample which had been disinfected with a solution of an organo-mercurial; that in the immediate background on the right was also sown with seed from the same sample which had in this case been treated with an organo-mercury powder fixed to the seed with separated milk.

(*Received* 9 *June* 1942)

(*b*) (*a*)
Fig. 1

Fig. 2

Fig. 3

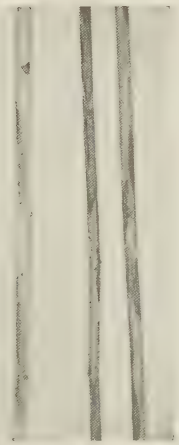

Fig. 4

Fig. 5

Effect of seed treatment on emergence of peas

By G. T. S. Baylis, R. S. Deshpande and I. F. Storey
Imperial College of Science and Technology, London

(With 3 Text-figures)

Experiments carried out during the early months of four consecutive years lead to the following conclusions: (1) high soil moisture accentuates the pre-emergence damping-off of peas; (2) a useful measure of disease control is obtained by dressing the seed with red cuprous oxide or an organic mercurial preparation. The addition of a sticker is undesirable with both fungicides; (3) whereas cuprous oxide has, more often than not, exceeded the mercurial preparation in giving increased emergence, the latter has the advantage of reducing the amount of seed-borne (*Ascochyta*) disease. In addition, the danger of an overdose is less with the mercurial preparation than with cuprous oxide; (4) the presence of a growth-promoting substance in a mercurial preparation gave no increased beneficial effect, as regards emergence, vigour of growth, or reduced liability to phytocidal damage.

Experimental work on pre-emergence damping-off of the garden pea led Hull (1937) to conclude that severity of disease was greatly accentuated by high soil moisture and that a certain measure of control could be obtained by dusting the seed with a commercial mercurial preparation. Baylis (1941) gave an account of the fungi concerned, the most important of which are *Pythium* spp. This paper describes work carried out each season from 1937 to 1941 and relates mainly to the use of seed dressings for control purposes.

The relevant literature was summarized in part by Hull. Later work was recorded by Brett *et al.* (1937), and Ogilvie *et al.* (1940) for England; by Davis (1938), Horsfall (1938), Walker *et al.* (1940) and McNew (1941) for America; by Hynes & Wilson (1939) for Australia; and by Neill (1938) and Hyde (1939) for New Zealand. There is general agreement that treatment of pea seed with organic mercurials or with copper preparations (more especially with red cuprous oxide) often leads to an increased stand, especially when the soil is wet and when the variety of pea is wrinkled-seeded. On the other hand, there is a risk of phytocidal damage when the treatments are applied under dry soil conditions.

The method of experiment adopted in this work was essentially as described by Hull (1937). The standard plot consisted of a row of 50–60 seeds sown 2 in. apart. For each date of sowing, the various treatments were randomized in compact blocks, with usually a fourfold replication. In addition, some experiments were carried out with pots or boxes, in a warm greenhouse or in cold frames. Soil temperature at a depth of about 2 in. was recorded. Soil moisture was determined at 7- or 10-day intervals as follows: one sample of the top 3 in. of soil was removed at each of four fixed points with a tubular sampler, these cores were mixed and the amount of water in the composite sample estimated in triplicate by drying at 105° C.; the results are expressed as percentages of the saturation capacity (Keen & Raczkowski, 1921) which varied between 50 and 65% according to the soil used.

Throughout the work the following variants were considered:

(*a*) *Date of sowing.* Sowings were made in each year as soon as the ground permitted, and continued at intervals until April.

(*b*) *Nature of soil.* Three types were available: (1) light sandy loam, (2) good medium loam, (3) low-lying heavy loam with clay subsoil which normally had a high water-table in the spring months.

(*c*) *Variety.* For the first three season vars. Pilot (round-seeded) and Gradus (wrinkled-seeded) were used. In 1940 and 1941 further varieties were tested.

(*d*) *Dosage of seed protectant.* Hull varied the dosage of organic mercurial (ceresan) between the limits 0·25 and 1% of the weight of seed, with little resultant effect on protective action: the larger doses were not carried by the seed. In order to increase the load of dust carried, experiments were made with various stickers, and of these 20% soft soap was adopted. The seed was treated for 5 min. with this solution, allowed to dry, and then shaken up with a measured quantity of dust. By this means a substantial increase of the load of seed dressing is obtained, e.g. with dressings of 0·5 and 1% ceresan, the amounts retained were 0·48 and 0·76% respectively. The adhesiveness of red cuprous oxide (cuprocide) is greater than that of the mercurial, so that in general it was applied directly to the dry seed.

Effect of soil temperature and soil moisture on emergence

Hull's conclusion that soil temperature was less important than soil moisture in its effect upon intensity of disease was confirmed by Baylis (1941). The following experiment illustrates the striking effect produced by high soil moisture immediately following seed sowing, and confirms similar experiments by Jones (1931), Horsfall (1938) and Ogilvie *et al.* (1940).

Seed boxes were filled with ordinary compost and sown with Pilot and Gradus (40 per box). These were watered either immediately on sowing or at intervals of 1, 2, 3 days afterwards. Each treatment was replicated four times. The experiment was carried out in frames during July 1939 (Table 1). Watering immediately after sowing markedly depressed emergence, and a steady improvement was shown as the date of watering was postponed. There appears to be a critical period during the course of germination when high soil moisture is very deleterious. The duration of this period depends upon the rate of germination, which is related to soil temperature. Under conditions of open ground, soil moisture and temperature are liable to considerable fluctuation, especially in the uppermost few inches of the soil; hence it is doubtful whether data of soil conditions and of emergence can be correlated in detail until further information is available regarding the period of maximum susceptibility of seedlings.

TABLE 1. *Effect of time of watering upon emergence of peas*

Treatment	% emergence	
	Pilot	Gradus
Boxes watered immediately after sowing	21·5	3·8
Boxes watered 1 day later	51·9	27·5
Boxes watered 2 days later	60	44·4
Boxes watered 3 days later	80	76·3

EFFECT OF SEED DRESSINGS UPON EMERGENCE

As the details of experiment varied somewhat from year to year each year's results will be presented separately. Wherever possible the results were submitted to an analysis of variance, the 5 % point being taken as the limit of significance.

1937. The period over which sowings took place (1 Feb.–5 Apr.) was characterized by heavy rainfall and absence of frost. The average weekly ground temperature (9 a.m.) remained constant at about 4° C., while soil moisture fluctuated between 48 and 65 % of saturation, and did not fall appreciably until the latter part of April. Thus, throughout the whole period when germination was taking place, soil moisture was abnormally high and, therefore, highly favourable to development of the disease. Nevertheless, emergence of untreated batches of the two experimental varieties, Pilot and Gradus, was surprisingly good, ranging between 65 and 86 %, and 75 and 91 % respectively. It was remarkable to obtain stands of the wrinkled-seeded variety Gradus equivalent to 80–90 % of seed sown in early February. The probable explanation of these unusual results is that the prevalence of the *Pythium* spp. concerned was low for the time being. It is known from work in progress that the maintenance of certain soil conditions markedly reduces the prevalence of *Pythium* spp. and other soil fungi, but it is not yet possible to explain why this result should have occurred in the early months of 1937. It may be noted in this connexion that Walker *et al.* (1940) stated that 'the conditions which favour cotyledon decay during germination occur periodically, but not necessarily consistently, under average Wisconsin conditions'. It thus appears that some factors, other than the prevailing environmental conditions, play a part in determining the intensity of disease.

Only one treatment was carried out in 1937, viz. dusting with 0·5 % ceresan with 20 % soft soap as sticker. As emergence of the controls was exceptionally high, thus leaving little room for improvement, the full data need not be given. A summary of the results of the 14 comparisons made for each variety is given in Table 2, where the results from sowings in heavy and light soil are combined. On the whole, therefore, the treatment was beneficial though there was evidence of phytocidal damage in some cases. Damage to the primary root, as noted by Hull, was observed, but this was not sufficiently serious to retard the growth of the plants aboveground.

TABLE 2. *Effect of seed treatment upon emergence*

	Treatment better than control	No difference	Treatment inferior to control
Pilot	10 (3 signif.)	1	3
Gradus	10 (1 signif.)	0	4

1938. Soil conditions in the first 4 months of 1938 were in marked contrast with those of the preceding year. Soil temperature was lower for the second half of February but higher throughout March. The chief difference, however, lay in moisture relationships, for the early spring of 1938 was as unusually dry as its predecessor had been unusually wet. As a result the light soil fell rapidly below 50 % of saturation at the end of February and became very dry by the end of April. A similar trend was shown by the heavy soil, but more gradually.

The seed treatment adopted was the same as in 1937, viz. 0·5 % mercurial with 20 % soft soap solution as sticker. In accordance with the trend of soil moisture, emergence of the controls improved in the later sowings. Significant increases in stand by the mercurial seed dressing were obtained in the earliest sowings only; later a more or less definite phytocidal effect was shown. The data for var. Pilot are contained in Table 3. Var. Gradus, which was also used, reacted in a similar manner, but as the seed was of low germination capacity (germination 70 %; healthy germination 29 %) the figures of emergence were poor throughout and are omitted from the table. The outstanding result was the phytocidal damage shown in later sowings, and associated with dry soil conditions.

A number of small-scale experiments was carried out in which various dosages of mercurial and of red

cuprous oxide (cuprocide) were used, all being applied without sticker. The results were in general agreement with those of Table 3, viz. in the earliest sowing (8 Feb.) a significant increase in emergence, greater with cuprous oxide than with the mercurial, resulted from the treatments; in sowings of 28 Feb. the effect was negligible, and in sowings of 14 Mar. all the treatments depressed emergence.

1939. Sowings were made at five dates on light and heavy land with vars. Pilot and Gradus. Seed

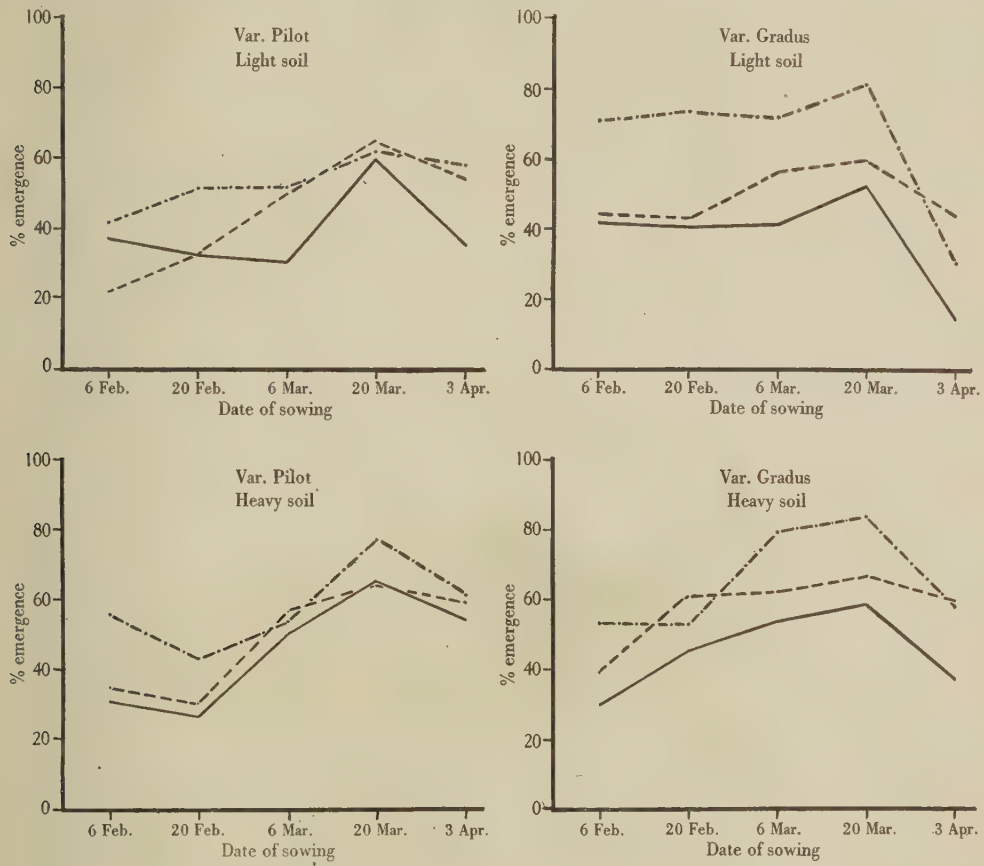

Fig. 1. Effect of seed treatment on emergence of peas in light and heavy soil.
——— Control. — — — Ceresan. —·—·—· Cuprous oxide.

Table 3. *Effect of seed treatment upon emergence of Pilot pea*

Date of sowing	Treated	Control	Difference
	Light soil		
31 Jan.	70·0	55·5	+14·5*
14 Feb.	59·5	70·0	−10·5
28 Feb.	77·0	82·0	− 5·0
14 Mar.	73·0	86·0	−13·0
	Heavy soil		
31 Jan.	73·5	60·5	+13·0
14 Feb.	67·0	73·5	− 6·5
28 Feb.	67·0	78·0	−11·0
14 Mar.	75·0	93·0	−18·0†

* Significant differences in heavy type.
† Based upon two replicates only.

of the former was reported by the Official Seed Testing Station, Cambridge, as being of rather low quality, having a germination capacity of 70 % and being rather heavily affected with marsh spot. Gradus seed was of normal quality, giving 86 % germination. Two treatments were compared: (1) 0·2 % cuprous oxide, without sticker, (2) 0·5 % mercurial with 20 % soft soap solution applied as described above (p. 19). Both treatments in general increased emergence, and in the majority of cases cuprous oxide proved to be superior to the mercurial. Fig. 1 gives the results obtained for all five sowing dates.

The notable feature shown by all the graphs is the high rate of emergence of the sowings on 20 Mar., followed by a sharp drop in those of 3 Apr. The

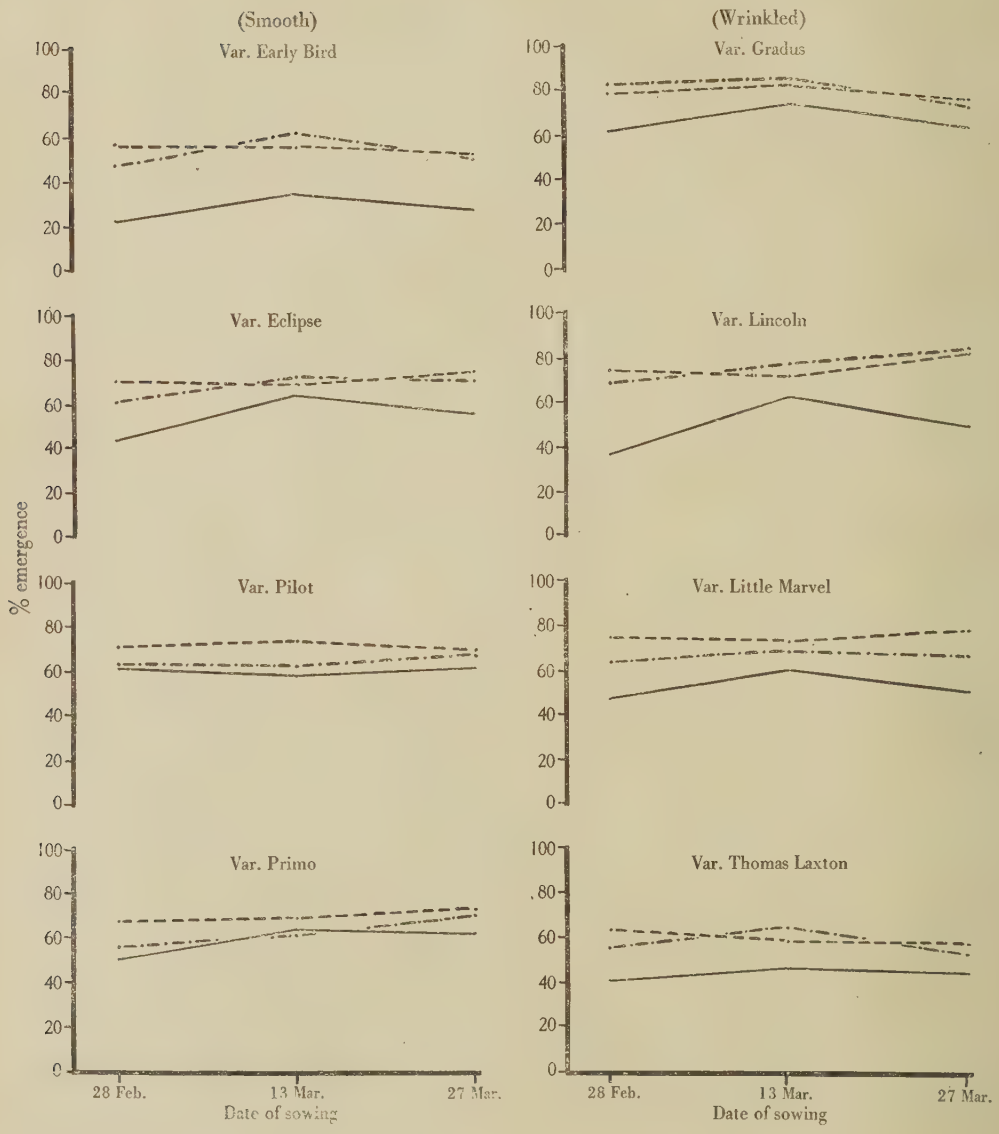

Fig. 2. Effect of seed treatment on emergence of eight varieties of pea.

——— Control. — — — Ceresan. —·—·—· Cuprous oxide.

records of rainfall and of soil moisture give the following comparisons: (1) The sowing of 20 Mar. was made at a time when soil moisture had been falling for some weeks, and the rainfall in the succeeding 10 days amounted to only 0·52 in. (2) The sowing of 3 Apr. was made near the peak of a sharp rise in the curve of soil moisture, and 1·47 in. of rain fell in the 4 days following sowing. As soil temperature was about 3° C. higher in the week following 3 Apr., one is justified in correlating the good emergence obtained in the sowing of 20 Mar. with low soil moisture, and conversely for the later sowing. Fig. 1 also illustrates that the response of Gradus to fungicidal treatments is generally more marked than that of Pilot. This difference is more clearly seen when the ordinates represent emergence in terms of germinable seed and not, as in Fig. 1, of seed sown. Altogether 20 comparisons of fungicidal efficiency are available and these are summarized in Table 4. The general result is, therefore, that 0·2 % cuprous oxide applied to the dry seed was superior to 0·5 % ceresan applied with sticker. Apart from two cases with ceresan (in one of which the difference was negligible) there was no evidence of phytocidal damage.

Table 4. *Effect of seed treatment upon emergence*

	Increase	No difference	Decrease
Cu_2O *v.* control	20 (14 signif.)	0	0
Ceresan *v.* control	17 (6 signif.)	1	2 (1 signif.)
Cu_2O *v.* ceresan	15 (10 signif.)	0	5

Similar results were obtained with sowings (Mar.–Apr.) in seed boxes, half of which were maintained in a warm greenhouse and half in a cool shed. It was also found that a good measure of control was obtainable by adding a certain amount of formalin dust (15 g. formalin + 85 g. absorbent) to the seed drill. Thus the addition of 6 g. formalin dust/10 ft. of seed drill raised the emergence of Gradus seed from 25 to 67 %. This method, though showing promise of efficiency, has the demerit of inconvenience. Direct treatment of seed with this dust was harmful.

1940. The number of varieties under experiment was extended as follows:

Round-seeded	Wrinkled-seeded
Early Bird (91*)	Gradus (98)
Eclipse (96)	Lincoln (99)
Pilot (80)	Little Marvel (99)
Primo (83)	Thomas Laxton (82)

* Percentage germination.

Attention was also paid to the effect of seed dressings containing hormones, and to the control of seed-borne disease (*Ascochyta* spp.). Two treatments, both given without sticker, were used: (1) cuprous oxide 0·25 %; (2) ceresan 0·5 %. On account of long-continued frost, very early sowings could not be attempted, and all the sowings, three in number, were made on light land. Both treatments, at all dates of sowing, almost invariably improved emergence, more strikingly with some varieties than with others. Analysis of the twenty-four comparisons available is given in Table 5. The general result is that 0·5 % ceresan applied to the dry seed was somewhat better than 0·25 % cuprous oxide similarly applied.

Table 5. *Effect of seed treatment upon emergence*

	Increase	Decrease
Cu_2O *v.* control	23 (16 signif.)	1
Ceresan *v.* control	24 (18 signif.)	0
Cu_2O *v.* ceresan	7	17 (3 signif.)

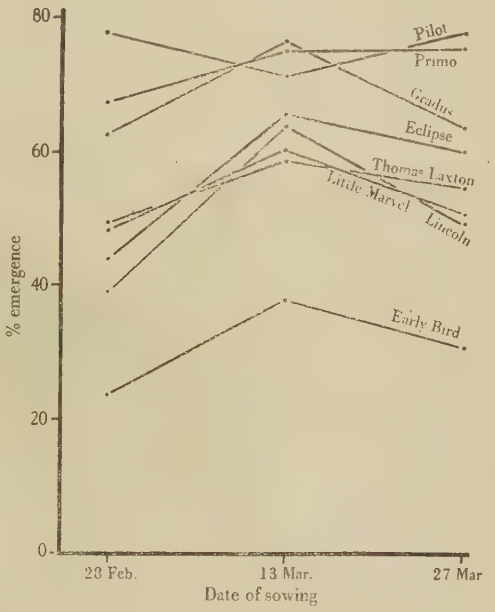

Fig. 3. Emergence of untreated seeds of eight varieties of pea expressed as percentage of germinable seeds present.

The data of emergence of the controls are given in Fig. 3 in terms of percentage of germinable seed, and these do not fully confirm the popular view that round-seeded varieties are more resistant to pre-emergence damping-off. Of the four round-seeded varieties used, Pilot and Primo gave very good emergence, Eclipse was next and Early Bird was lowest. Of the four wrinkled-seeded varieties, Gradus gave very good emergence but the others showed intermediate results. While it may broadly be true that round-seeded varieties are more resistant, it would seem that germination capacity of the sample used, as expressed not merely by percentage of viable seed

but also by vigour and healthiness of germination, may exert a considerable modifying effect.

It has been claimed by Croxall & Ogilvie (1940) for peas, and by certain workers for other crops, that the incorporation of a growth hormone in a seed dressing gives beneficial results, e.g. greater emergence and vigour of growth with resultant increased yield, over and above those due to the seed dressing alone. Examination by Templeman & Marmoy (1940) failed to confirm such an effect with a number of crops, not, however, including peas. To test this point, sowings were made on two dates on light and heavy land. Two seed lots were chosen, one having a high (Gradus 98%) and the other having a low (English Wonder 68%) germination capacity. The treatments, given in triplicate, were as follows: (1) cuprous oxide 0·25%; (2) ceresan 0·5%; (3) a hormone-containing mercurial 0·5%; (4) control. In no case was a sticker used. All three fungicidal treatments gave good results, especially with var. 'English Wonder' in which emergence of the controls was poor. The improvement resulting from the seed treatments was substantially the same for all, and there was no suggestion that any advantage had accrued from the incorporation of a growth hormone in one of the seed dressings.

In a further experiment a direct comparison was made of two mercurial seed dressings, one with and one without growth hormone. Neither caused a visible check to growth when the seed was treated in the dry state at the rate of 0·5% of seed weight. After treatment of the seed with soap solution in the manner described on p. 19, and with the dose already stated, a check to growth was noticeable, but it was no greater with the one dressing than with the other. No evidence therefore was obtained that the inclusion of a growth-promoting substance reduces the liability to phytocidal damage.

A further subsidiary experiment was carried out to test the relative efficiency of cuprous oxide and ceresan for the control of seed-borne disease of the *Ascochyta* type. Two lots of seed, Lincoln and English Wonder, which were known to carry infection, were used for this purpose. The treatments were: (1) cuprous oxide 0·25%; (2) ceresan 0·5%; (3) control. Six plots (10 × 10 ft.), each containing twelve rows of sixty seeds, were used: in each, six rows were devoted to one treatment of one variety, the other six to the same treatment of the other variety. This simplified arrangement was adopted to diminish the risk of the experiment being vitiated by secondary spread of the disease. The data of emergence and of number of plants showing primary infection with *Ascochyta*, the latter expressed as percentage of emerged plants, are given in Table 6.

The effects of seed treatments upon emergence are in general agreement with those of Table 5. The outstanding effect upon infection by *Ascochyta* is the good control given by ceresan for var. English Wonder. In the absence of complete randomization, exact statistical assessment was not attempted, but as the degree of infection showed a marked uniformity in the various rows under each treatment, the substantial nature of the result cannot be doubted.

1941. In the main experiment of this year, vars. Primo, Pilot, Gradus and Lincoln were sown under the following treatments: (1) cuprous oxide 0·25%; (2) cuprous oxide 0·25%, with soap sticker; (3) ceresan 0·5%; (4) ceresan 0·5%, with soap sticker; (5) control. Sowings were made on light, medium and heavy land, and at two dates for each. In this somewhat complicated experiment, only a twofold replication of plots under one set of conditions was possible. As regards the comparative merits of the treatments, the same trend was shown in all three types of soil and, therefore, in order to simplify presentation and to enable the results to be

TABLE 6. *Effect of seed treatment upon emergence and upon infection by* Ascochyta

	Var. English Wonder		Var. Lincoln	
Treatment	% emergence	% *Ascochyta*	% emergence	% *Ascochyta*
0·25% cuprous oxide	60·7	12·5	66·3	1·6
0·5% ceresan	63·8	2·6	78·7	1·4
Control	52·6	11·7	57·5	2·5

TABLE 7. *Effect of treatment on emergence of peas*

	Increase	Decrease
Ceresan *v.* control	8 (7 signif.)	0
Cuprous oxide *v.* control	8 (7 signif.)	0
Ceresan *v.* cuprous oxide	2	6 (1 signif.)
Ceresan *v.* ceresan with soap	6 (1 signif.)	2
Cuprous oxide *v.* cuprous oxide with soap	8 (4 signif.)	0

treated statistically, similar treatments in the three soil types are grouped, thereby giving a sixfold replication. This allowed of an eightfold comparison (four varieties at two sowing dates) of the various treatments (Table 7).

The main conclusions are as follows: (1) Ceresan 0·5%, and cuprous oxide 0·25%, markedly increased emergence, and this effect was greater with cuprous oxide than with ceresan. (2) The addition of a sticker was deleterious for both protectants, more so with cuprous oxide than with ceresan.

The magnitude of the effects obtained is shown in Table 8—which gives the emergence of all four varieties on the different types of soil at two sowing dates. Only the data for controls and for cuprous oxide treatments without sticker are included, these giving in general the worst and the best emergence respectively. Table 8 shows that, in general, (1) emergence of untreated peas is better at the later sowing date and that this effect is most pronounced in the

heavy soil, (2) the relative improvement due to seed treatment is greatest in the early sowing, (3) the wrinkled-seeded varieties, Gradus and Lincoln, give the best response to seed treatment. The improvement is most marked with the early sowing in heavy soil, where very poor stands were converted into reasonably good ones by seed treatment.

In a subsidiary experiment, the behaviour of good seed of a wrinkled variety (Gradus 90 % germination) was compared with that of poor seed of a round variety (Early Bird 69 % germination). A sixfold replication of plots was arranged in medium loam (Table 9). Emergence of untreated Gradus seed was good, so that any improvement arising from seed treatment was necessarily small. On the other hand, substantial improvements were shown in the Early Bird series, in which emergence of the controls was poor. On the whole, ceresan was superior to cuprous oxide in this experiment. Table 9 illustrates a point already referred to, viz. that while improved emergence from seed treatments is more frequently shown by the wrinkled-seeded varieties, the quality of seed, as measured by percentage germination, exerts a considerably modifying effect.

Table 8. *Emergence of peas under different conditions* (*soil, date of sowing, seed treatment*)

		% emergence					
		Sandy soil		Medium soil		Heavy soil	
Variety	Treatment	1st sowing	2nd sowing	1st sowing	2nd sowing	1st sowing	2nd sowing
Pilot	Control	63	84	59	63	37	72
	Cu_2O	74	82	77	71	65	86
Primo	Control	59	63	58	61	46	73
	Cu_2O	69	82	62	65	68	84
Gradus	Control	59	83	71	70	34	86
	Cu_2O	86	87	83	89	75	89
Lincoln	Control	25	53	41	52	11	51
	Cu_2O	70	70	54	62	58	69

Table 9. *Effect of seed treatment upon emergence*

		% emergence	
Variety	Treatment	1st sowing	2nd sowing
Gradus	Control	74·4	80
	0·25 % Cu_2O	79	86
	0·5 % ceresan	88·7	86·2
Early Bird	Control	21·3	47·7
	0·25 % Cu_2O	37·6	60
	0·5 % ceresan	51·7	68·3
Sig. diff. at 5 % point		3·7	4·2

A further experiment, with fivefold replication, was carried out in which the efficiency of cuprous oxide as seed dressing (0·25 % of seed weight) was compared with that of formalin dust distributed along the seed drill. The varieties used were Gradus and Eclipse and sowings were made on two dates. Quantities of 5 or 10 g. formalin dust/12 ft. of seed drill gave results very similar to those of the cuprous oxide seed dressing. When the quantity of formalin dust was increased to 20 g./12 ft. of drill, definite phytocidal damage was shown in all sowings.

Discussion

These experiments, which cover a period of five seasons, generally confirm the views now held concerning the occurrence of pre-emergence damping-off in peas and establish the value of certain fungicidal treatments. There are still important gaps in our knowledge of this disease problem, and it will be useful to indicate these.

The etiology of the disease is not simple. There is now a considerable body of evidence that *Pythium* spp. play a prominent part (Ogilvie *et al.* 1940; Baylis, 1941) and that *Fusarium* spp. may also be very destructive to peas germinating in soil (Baylis, 1941). It does not follow, therefore, that the organisms which are primarily responsible for the disease are the same in different soils or even in the same soil under different conditions. Thus, the dependence of disease intensity upon high soil moisture, a result which was obtained in soils inoculated with *Pythium* spp. and also in naturally infected soils, cannot be applied if other organisms (e.g. *Fusarium* spp.) are concerned. The effect of environmental conditions on the intensity of disease caused by the latter is unknown.

The disease intensity is the resultant of a suitable environment, and the presence of an effective inoculum potential of the parasite. Thus, if the parasite should be absent, the disease would not occur even under the most favourable environmental conditions. This explanation is suggested for the results obtained in the spring of 1938 when, in spite of unusually wet soil, very little disease was shown by a susceptible variety. That the concentration of a parasite in the soil should fluctuate is not a new idea, but very little is known about the determining factors.

Records of soil temperature and moisture were taken throughout the experimental period in each

year. No correlation between the emergence data and the records of soil temperature could be established and this is in accordance with Hull's (1937) findings. On the other hand, high soil moisture was stated by Hull and by certain American workers to favour the disease and this view was confirmed in several instances, e.g. in experiments described on pp. 19 and 21 above. Furthermore, as the season (Feb.–Apr.) progresses, soil moisture as a rule falls, and with this there is a tendency for the emergence of untreated seeds to improve in successive sowings. While certain broad conclusions are thus possible, the detailed records of emergence cannot be interpreted on the basis of soil moisture curves. It may be doubted whether the latter, based as they were on 7- or 10-day determinations, record sufficiently accurately the conditions to which germinating peas were subjected.

On the relative merits of cuprous oxide and an organic mercurial (ceresan), no clear-cut decision can be made. Many successful results were obtained with both seed dressings. Although cuprous oxide more often gave the better result, there were experiments in which the advantage was distinctly with ceresan. The addition of a sticker was found to be harmful for both fungicides, thus indicating the danger of an overdose. In this respect a certain advantage accrues to the ceresan in that its capacity of adhering to dry seed is low and for this reason phytocidal damage is less likely to occur even when an excess of the fungicide is used. A further advantage of ceresan is that it exceeds cuprous oxide in the control of seed-borne diseases due to *Ascochyta*.

The present paper supports the view that the wrinkled-seeded varieties of pea are more susceptible to pre-emergence damping-off than the round-seeded ones, but the rule is not absolute. The germinative vigour of the seed used appears to exercise a modifying effect, and it is not surprising that the best results of fungicidal control are often obtained with seeds of rather low quality.

In spite of the lack of knowledge in certain directions the main practical result is clear. Soil conditions are liable to exist, especially in the earlier months of the year, which are inimical to the emergence of peas. By the simple process of dusting the seeds with certain fungicides a substantial increase of stand may often be produced.

The writers wish to express their thanks to Prof. W. Brown, F.R.S., for much advice and assistance during the progress of the work; to the Official Seed Testing Station, Cambridge, and to the Plant Pathological Laboratory of the Ministry of Agriculture and Fisheries for carrying out germination tests on peas; to Messrs Sharp and Co., Ltd., Sleaford, for supplying some of the seed used; and to the Agricultural Research Council for a grant in aid during the later years of the research.

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(*Received* 22 *April* 1942)

An investigation into the 'stripe' disease of narcissus

II. Experiments on the virus agent and its spread

By John Caldwell and Ian W. Prentice
Department of Botany, University College, Exeter

(With 3 Text-figures)

Experiments on the transmission of the 'stripe' disease of the narcissus are described. It was found that the disease is not seed transmitted but that it is transmissible by grafting and by juice inoculation with the use of an abrasive such as carborundum powder. Under field conditions transmission of the disease takes place above ground and not through the roots. The identity of the vector has not been established. The effect of the disease in reducing bulb growth and vigour has been examined. Controlling the spread of the disease by roguing is recommended.

In 1938 Caldwell & James reported that 'stripe' disease of narcissus was characterized by a fairly wide range of symptoms, and suggested the existence of a virus complex responsible for the symptom expression, and of varying intensity of action of the different components under different conditions. This paper describes the results of further investigations which have been carried out by the writers of this paper and by A. L. James, who was succeeded by I. W. Prentice.

Symptom expression: varietal reactions to infection

Chlorosis. This is the commonest reaction to infection in the field and is most marked when the leaves are 3–4 in. long. This occurs in the early part of the year and the intensity of the chlorosis is associated, apparently, with the temperature since warm weather seems to result in masking of the chlorosis: e.g. var. Princeps shows marked chlorosis in garden plots but hardly recognizable marking when grown in a glasshouse at about 50° F. Bulbs of a *Narcissus triandrus* hybrid were planted in soil in pots and left outside till the leaves were 2 in. high; the pots were then brought into the glasshouse and left there while the leaves grew another inch; they were then taken outside again while the leaves grew yet another inch, when they were returned to the house. All the *triandrus* group show marked chlorosis under normal conditions, but in this instance the leaves were clearly 'zoned', chlorotic bands developing while outside and normal green tissue in the heated glasshouse. This masking of the chlorotic markings has been noted in plants growing under field conditions. It is difficult to recognize mosaic in narcissus plants in late spring and early summer, unless proliferation is a regular system of the disease. Masking may be pronounced all through the season with the result that selection of stocks for inoculation is difficult.

Marked chlorosis has been noted in Golden Emperor, M. J. Berkeley, Minister Talma, The First, Winter Gold, Princeps, Helios, Princess Victoria, and in a *N. triandrus* hybrid.

Proliferation. In addition to chlorosis proliferation is often found. This is an outgrowth of the mesophyll tissues under or through the epidermis (Caldwell & James, 1938) and it is not masked by high temperature. Plants of Princeps which showed slight chlorosis out of doors showed little chlorosis but marked proliferation when taken into the glasshouse. Under field conditions proliferation becomes more marked in mid-season, and this may be associated with the higher temperatures then prevailing and probably is associated with the change in symptoms noted above for Princeps. The varieties which show marked proliferation are Mrs E. H. Krelage, Princeps, Croesus, and Phyllida: Sir Watkin also shows it under certain conditions, and it may be severe, at times, but the variation in symptoms is great. Our observations suggest that proliferation and chlorosis may be induced by the same virus, the response on the part of the plant being conditioned by varietal and environmental differences. This view is held by Haasis (1939).

Distortion. Distortion accompanied by severe local chlorosis has been noted in King Alfred, The Marquis, Victoria, and Helios, and occasionally in Lord Kitchener, Phyllida, and Buttercup. The incidence and severity of the distortion is extremely variable. Hodson (1928) suggested that the sickle-form adopted on occasion by the leaves of King Alfred was associated with a special form of the disease which he called 'King Alfred' stripe, but this variety may show normal chlorosis and chlorosis with distortion. In cases where sickle leaf occurs in the absence of marked chlorosis in King Alfred there is often evidence of bulb-fly damage or of extreme mite infestation.

Corrugation. In diseased plants of some varieties,

notably Beersheba, Damson, Bath's Flame, and Sunrise there is a marked corrugation of the leaf which is caused by the different rates of growth in the mesophyll and epidermal tissues. It is not certain that corrugation is an infallible sign of this infection but it is probably associated with it.

The symptom expression of mosaic disease in narcissus shows considerable seasonal variation, particularly noted in Magnificence, Sulphur, King Alfred, Amir, Damson, Beacon, Sunrise, Dragoon, Red Chief, and Legacy which in some seasons may look healthy or show only the very faintest marking. In 1937–8, plants of different varieties showing clear markings of a mosaic type were collected and added to our collection of diseased bulbs. Three of the varieties have since shown very indistinct symptoms, Sarchedon showing faint mosaic marking whilst Beersheba and St Agnes, might, in the last two seasons, have passed as healthy. Masking and variation have been studied because of the difficulties which can arise in continuous experimentation when a variety is noted by one person as infected in one year and by a second person as healthy in the next year. The authors together, in 1939, collected plants of Dick Turpin which they were both satisfied showed pronounced mosaic markings: these plants for the last 2 years have been noted as healthy.

Break in flowers. Some varieties of yellow narcissus regularly show a white 'break' in the flower colour, which is more usual in the perianth than in the corona. It has been noted in the perianth of Winter Gold, M. J. Berkeley, Golden Emperor, Gog, Minister Talma, The First, *N. pallida praecox*, Magnificence, Wrestler, Sulphur, Slievenamon, King Alfred, Princeps, Victoria, Helios, Princess Victoria, Sir Watkin, Bath's Flame, Husky, and Van Sion, and also in the corona of M. J. Berkeley, Minister Talma, The First, Sulphur, Slievenamon, Princeps, and Victoria.

Experiments on spread of 'stripe' under field conditions

In September 1937 the manner of spread of the disease in the field was examined. Two plots of healthy bulbs, each plot 5 ft. square, were prepared and around them were planted rows of diseased bulbs. Plot A was sprayed weekly with nicotine insecticide during the season 1937–8. Plot B was enclosed in a wooden frame sunk into the ground for a distance of about 2 ft. the upper edge of the frame being on ground level: this plot was not sprayed or treated in any way (see Fig. 1). During 1936–7–8 the bulbs remained free from infection, but in 1938–9 disease appeared, 3% of the sprayed bulbs in plot A showing mosaic markings and 14% of the unsprayed bulbs in plot B.

Spread of infection in plots of Sir Watkin narcissus

It became clear that different varieties of narcissus showed different reactions to infection, and varying intensity of symptom expression. Some varieties, notably Barrii conspicuus, were resistant, as were the *N. poeticus* and certain other groups. Sir Watkin appeared to be a variety which showed clear, well-developed symptoms, and for this reason was chosen for many of the later experiments; in the earlier experiments healthy bulbs of several varieties had been used.

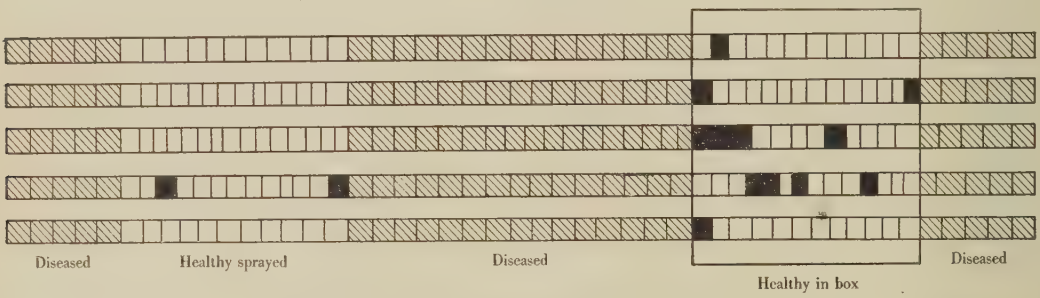

Fig. 1. Lay-out of experiment with isolated narcissus to ascertain if spread was above or below ground. The black squares indicate diseased bulbs in second year.

In autumn 1936 a plot of Sir Watkin was laid down containing 15 rows each of 20 bulbs of a mixed collection previously grown on a commercial scale. In the 1937 season 98 virus-infected bulbs were removed from the plot, roughly one-third of the total. In 1938, 83 bulbs were noted as infected, being 41% of the total at the beginning of the season: 50% of the bulbs infected adjoined the gaps left on the removal of the infected bulbs in the previous year. In 1939, 123 bulbs were noted as infected, leaving only 58 bulbs of the original 300 healthy in the fourth season. The spread, therefore, can under certain circumstances be rapid. In this experiment each bulb or the progeny of each bulb is counted as one, no account being taken for the purposes of the count of the multiplication of the bulbs by division each year (see Table 1). When the plants were lifted for the final count in 1939, in six instances the progeny of a single bulb at the beginning of the experiment was 'mixed' in health, i.e. some of the new plants were infected and others were healthy. This indicates

TABLE 1. *Numbers of healthy and infected plants of Sir Watkin narcissus*

Row	1937 Diseased plants removed	1938 Healthy	1938 Diseased	1939 Healthy	1939 Diseased
1	13	—	6	—	6
2	14	2	2	—	4
3	5	8	5	4	9
4	7	5	8	1	12
5	9	5	4	5	4
6	9	3	5	2	6
7	3	8	7	5	10
8	7	5	7	1	11
9	3	6	10	3	13
10	10	7	3	5	5
11	3	11	5	5	11
12	7	7	5	3	9
13	3	8	6	6	8
14	2	14	4	12	6
15	3	9	6	6	9

that the infection probably passes in the aerial parts, a conclusion which was indicated by the experiment on spraying and isolation noted above.

The lifted bulbs were sorted into groups. The 123 infected plants gave 180 single-nose bulbs and 146 double-nose (55 and 45%). The 58 healthy plants gave 160 single-nose and 90 double-nose (64 and 36%). The bulbs were then sorted by weight (see Table 2). As compared with the healthy bulbs a larger proportion of virus-infected bulbs falls in the lower weight classes and a smaller portion in the higher weight classes, which suggests that the infected bulbs multiplied less rapidly and produced smaller daughter bulbs than did the healthy.

A second investigation was begun in September 1937. No infected bulbs were removed, but the position of the healthy and diseased bulbs was marked. Fig. 2 shows the changes in the plot in the season 1937–8 and 1938–9.

Third and fourth plots were laid down in 1939–40 to investigate the spread of infection along and across the rows. The third plot was in the form of a cross, while the fourth plot was laid down with healthy bulbs in one, two, three and four rows in association with the rows of diseased plants (see Fig. 3 *a*, *b*). In neither the 1940 nor the 1941 season were conditions favourable to infection in the field and only a few infections were noted: the experiments are in progress.

These conclusions confirm the findings of Haasis (1939), who stated that he found it impossible to carry out experiments in plots by reason of the

TABLE 2. *Classification of bulbs by weight*

Single-nose wt. in g.	Diseased	Healthy	Double-nose wt. in g.	Diseased	Healthy
Under 15	65% { 76	39	Under 25	66% { 41	9
15–17	19	12 } 44%	25–30	30	6 } 33%
18–20	21	20	31–35	22	15
21–25	33	24	36–40	22	14
26–30	17% { 18	26	41–45	22% { 11	8
31–35	6	14 } 40%	46–50	10	13 } 50%
Over 35	7	25	Over 50	10	25
	180	160		146	90

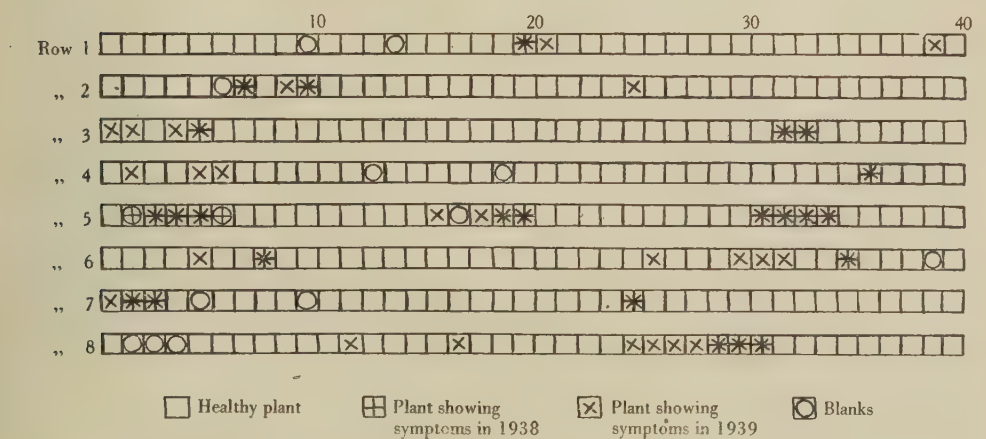

Fig. 2. Plan of plot of Sir Watkin narcissus showing spread of infection.

amount of accidental infection which occurred. We have not encountered this difficulty, and, on the other hand, prefer outdoor experiments, since masking of the symptoms is so easily induced by the higher temperature in glasshouses. We have had no accidental infection of our controls grown in plots in near proximity to inoculated bulbs, and spread

brackets (3rd and 5th columns) give the range of weights in each of the groups: in each case the healthy bulbs are heavier.

The lengths of the flower stalks from ground level to the foot of the gynoecium were also measured, and on the average the healthy stalks were from 0·5 to 7·0 cm. longer than were the infected. The range,

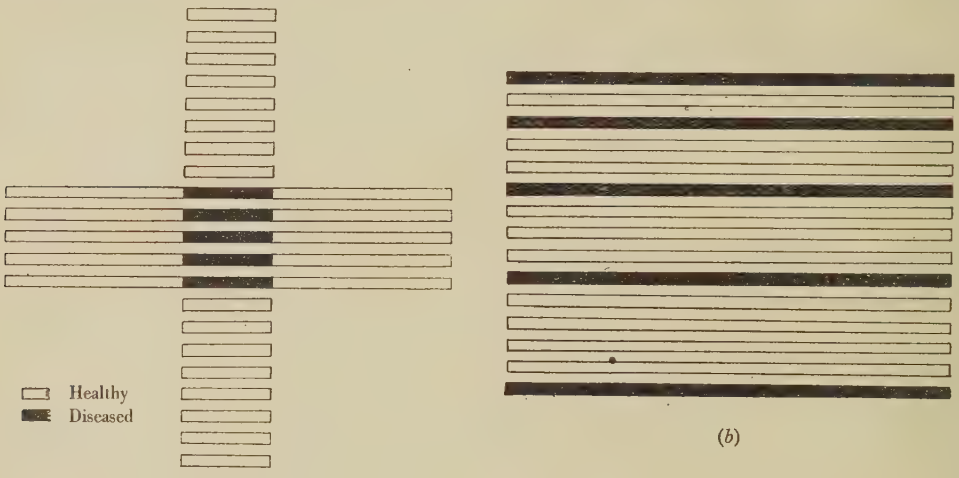

Fig. 3. Lay-out of 'spread' experiments: (*a*) to examine movement along and across rows; (*b*) to examine movement across adjacent rows.

TABLE 3. *Increase in weight of diseased and healthy bulbs (Sir Watkin)*

Original wt. (g.) July 1939	Healthy bulbs		Diseased bulbs	
	No. of bulbs	Wt. (g.) 1940	No. of bulbs	Wt. (g.) 1940
15–17	10	361 (26–47)	10	265 (12–42)
18–20	20	768 (21–53)	20	653 (15–45)
21–25	18	919 (39–62)	22	911 (28–54)
26–30	15	859 (33–70)	16	807 (32–60)
31–35	7	484 (54–80)	8	411 (33–69)
Over 35	8	664 (70–92)	7	471 (58–87)
	78		83	

TABLE 4. *Number of flowers open on successive dates*

Plants	22 Mar.	23 Mar.	24 Mar.	25 Mar.	26 Mar.	Additional buds
Single-nose bulbs (diseased)	10	22	13	23	6	2
Single-nose bulbs (healthy)	23	45	6	16	—	—
Double-nose bulbs (diseased)	6	19	9	36	26	4
Double-nose bulbs (healthy)	36	32	6	28	6	—

in the open in our plots has been very slight in our experiments on disease transmission.

YIELD TRIALS

To ascertain if multiplication and bulb production were on a smaller scale in infected than in healthy bulbs, a yield trial was set up in July 1939 with healthy and diseased bulbs. They were harvested in June 1940 and dried, and each was weighed in August after drying (see Table 3). The figures in however, was great, and the differences, though striking, were not statistically significant. The flowers of the healthy plants matured a few days earlier than did those of the infected plants; the differences were not great, but they were noted on many occasions. Table 4 gives the time of opening of flowers of four sets of plants. The total numbers of flowers were 76 from 75 single-nose infected bulbs, and 90 from 90 healthy; 100 from 50 double-nosed infected, and 108 from 55 double-nosed healthy bulbs.

In 1940–1 the increase in size of healthy and diseased bulbs was studied, using single-nose Sir Watkin bulbs. These were planted in September 1940, lifted in June 1941, dried, and weighed on 1 Aug. 1941 (see Table 5). The healthy bulbs of the 20–30 g. group show a greater increase in weight than do the virus-infected bulbs. A few of the bulbs in this experiment were attacked by the bulb-fly (*Eumerus tuberculatus*), and this probably accounts for the inconclusive results obtained with the 30–40 g. group. These results confirm the view of Haasis (1939) that virus-infected bulbs show a smaller increase than do healthy bulbs under the same conditions. In these experiments, also, it was noted that the healthy bulbs flowered a few days earlier than did the diseased.

TABLE 5. *Weights of healthy and diseased bulbs*

Bulbs	Wt. range g.	Total wt. g.	Wt. (g.) Aug. 1941	Increase %
20 healthy	30–40	683	1032	51
20 diseased	30–40	695	1100	58
50 healthy	20–30	1257	2330	85
50 diseased	20–30	1244	2015	62

The technique first adopted for disease transmission in the narcissus under laboratory conditions, viz. inoculation by means of a hypodermic syringe, is not very satisfactory. In the main, the inoculum appears to pass into the intercellular spaces of the mesophyll of the leaves and probably too little damage is done to the cells (Caldwell, 1932). Rubbing the leaves with infective juice and fine carborundum powder gives much better results, but even so the number of successful infections is relatively low. On average about 15% of successful inoculations are obtained, a very low 'take' as compared with, for example, tobacco mosaic. Another difficulty associated with narcissus disease is that a year elapses between inoculations and symptom development. Inoculations made into Tresserve produced symptoms of the Tresserve type regardless of type of symptom shown by the variety used as a source of inoculum. This suggests that the agent is a single virus rather than a virus complex as suggested by Caldwell & James (1938). Tresserve is characterized by a well-marked chlorosis and slight proliferation symptoms. The details of the source of inoculum and the symptoms of the plants from which the juice was taken are shown in Table 6. Juice from hyacinth, crocus, iris and tulip infected with mosaic disease did not cause infection in the narcissus, which indicates that the mosaic disease of narcissus is caused by an agent different from those of the other monocotyledons. An interesting point emerged from these experiments: when tulip plants were treated with the juice of narcissus plants or when grafts between the two plants were attempted, severe lesions were formed on the leaves of the tulip in the former case, or the bulb was killed in the latter. This is in accord with the findings of Haasis (1939).

TABLE 6

Source of inoculum	Symptoms of infected plant	No. inoculated	No. infected
Croesus	Marked proliferation	18	3
Princess Victoria	Chlorosis	17	3
Princeps	Chlorosis (or proliferation)	20	2
Sir Watkin	Chlorosis and proliferation	18	3
Lucifer	Do.	10	1
Helios	Chlorosis and distortion	7	3
Princeps (hypodermic method)	—	20	2
Hyacinth	Mosaic (hyacinth)	15	—
Control	—	40	—

INOCULATION AND INFECTION

There is no evidence of the spread of infection below ground, and the disease appears to spread in the aerial parts of the plants. During each season from 1936–7 seed has been saved from diseased plants, the flowers of which were pollinated by hand with pollen from diseased plants: the varieties include Sir Watkin, Phyllida, Mrs Krelage, Weardale Perfection, Advance Guard, and Princeps. Many hundreds of seedlings have been grown and studied, but in no instance has evidence been found of disease transmission through the seed.

In the season 1936–7 the transmission of the disease through grafts was studied, var. Princeps being used. A sector of about a third of each healthy bulb was removed with a sharp scalpel and replaced by a third of a diseased bulb, the two parts being strapped together with grafting tape. In 18 instances the grafts 'took' in that there was something approaching organic union between the stem plates of both bulbs. In all these cases the leaves of the previously healthy part of the bulb showed symptoms of disease in 1937.

DISCUSSION AND CONCLUSIONS

Numerous seedlings have been grown from seeds of infected narcissus plants, and all the evidence suggests that the agent of the disease is a virus that is transmitted in the aerial parts of the plants but not in the seed. The box experiment described above indicates that no transmission takes place through unbroken roots, which is supported by our observation that some of the offsets from a single bulb may be healthy while others are infected In these cases

the roots of all the bulbs were in the closest possible association. This conclusion supports the findings of Haasis but not those of McWhorter (1932), who reported root transmission. Haasis also found no evidence of seed transmission in the narcissus. Blanton & Haasis (1939) reported that a number of aphis vectors of narcissus mosaic virus exist in the U.S.A., and it is probable that some of these are the vectors in this country also. Normally very few insects are found on the narcissus, and there has been great difficulty in keeping aphides of various species alive on the experimental plants. Experiments with the bulb mite (*Tarsonemus* sp.) as a vector have so far given negative results. That spread does take place is clear from field observations and, on the whole, it is from infected plants to adjacent ones. The rate of spread varies with the season and also with the variety of narcissus, and some varieties are much more seriously affected by the disease than others.

Further complications are that symptoms are much more marked in some seasons than in others and, especially in the mosaic types, show great variation; and also the fact that symptoms are not recognizable till a year after inoculation. These make difficult the selection of healthy bulbs for experimentation since an apparently healthy bulb may show symptoms in the next season. We have grown supposedly healthy bulbs in isolation for two or three seasons before using them for experimentation.

From the practical aspect, the 'stripe' disease can be controlled fairly easily if careful roguing is practised. It is essential that roguing should be done systematically and at frequent intervals. As the season advances and temperatures rise masking of the mosaic symptoms becomes common, so the first inspections should be made as early as possible in the growing season. Again, it should be remembered that inoculations made during the growing season have not, in our experiments, yielded visible results until the next season. Only on a few occasions when inoculations were made into bulbs before the leaves had appeared were faint marks developed before the end of the current season, and the marks were so slight as to make it doubtful if they were, in fact, symptoms of disease. Roguing carried out as a routine practice is strikingly effective with even such a susceptible variety as Helios. Certain stocks of Helios which had been carefully and systematically rogued over a period of 3 years are now almost completely free from disease, whereas stocks of this and other varieties which had not been rogued are almost completely diseased. Other methods of control have been tried. One possible method which would be useful if it were effective, is the hot-water treatment of bulbs carried out to check eelworm infestation. Unfortunately, temperature inactivation of this virus seems to occur after exposures to between 70 and 75° C. for a period of 10 min., and the temperature at which bulbs are treated for eelworm infestation is 45° C. Another method confidently recommended by a grower as infallible consisted of soaking the bulbs during the dormant period in a 4% solution of formaldehyde: bulbs so treated were as diseased after the treatment as before.

Our thanks are due to the Agricultural Research Council for grants which made the investigation possible. We gratefully acknowledge also the kindness of many bulb growers and others who allowed us to visit their fields and who generously furnished us with specimens.

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(*Received* 11 *January* 1942)

A survey of potato aphides in the South-Western Agricultural Advisory Province

By L. N. Staniland, A.R.C.S., D.I.C.
Advisory Entomologist, Seale-Hayne Agricultural College, Newton Abbot, Devon

(With 9 Text-figures)

The results of a survey of aphis populations on potatoes in Devon and Cornwall, with special reference to *Myzus persicae* Sulz., during the years 1937–41 are given. One or more counts were made by the standard method at 107 centres to determine population index figures. The results are summarized in the form of a map. The highest index figure obtained during the 5 years was 609, and only on six other occasions was the figure of 100 exceeded; of these centres only one was situated in the area considered to be especially favourable to seed production, and this centre was a market garden. The distribution of the various species of potato aphides is discussed, together with their fluctuations from year to year; special reference is made to *M. persicae* and *M. ornatus*. Observations on the parasitism of *M. persicae* are described. The relationship of the proximity of winter hosts, altitude and of meteorological conditions, to the populations of *M. persicae* is discussed. The conditions for the production of seed potatoes in Devon and Cornwall are summarized. An account is given of those areas in Devon and Cornwall deemed suitable for seed production. A short account of the formation of a 'Seed Potato Growers' Association' in Devon and Cornwall has been added.

A survey of potato aphides in Devon and Cornwall was undertaken at the suggestion of Mr J. C. F. Fryer, Director of the Ministry of Agriculture Plant Pathological Laboratories. Surveys of potato aphides were carried out by Davies in North Wales (1934), and in Scotland (1939), and other aspects of the problem were also investigated by him (1935*a*, *b*, 1936, 1938). Dr Davies's work, some in collaboration with Dr T. Whitehead, dealt with districts having a moist and rather cool climate, and it was considered that it would be of especial interest and value to study the aphis populations on potatoes in the moist warm climate of Devon and Cornwall. Davies (1935*a*, *b*, 1936, 1938) showed that the principal conditions governing migration and multiplication of *Myzus persicae* Sulz. were temperature, humidity and wind velocity. Winged *M. persicae* flew readily at temperatures above 65° F., did not fly at all readily when the relative humidity reached 70%, and flight ceased at humidities above 80% or as soon as the wind velocity reached only 4 m.p.h. Thomas & Vevai (1940) carried out further work on aphis migrations and, in general, confirmed Davies's work. Davies also showed that meteorological differences do not account for the whole of the story, but that winter hosts are of great importance. Nectarines and peaches could not be regarded as accounting satisfactorily for the distribution of *M. persicae* in early summer. Subsequent examinations in North Wales showed that cruciferous plants, especially winter Brassicae, harboured large numbers of this species. He therefore emphasized the proximity of winter Brassicae to potatoes as an important factor in determining the initial aphis population on potatoes. Jacob (1941) carried out further work on the overwintering of *M. persicae*, and his work has confirmed and amplified that by Davies.

The present survey

The purpose of the survey was to ascertain whether the results on the distribution of *M. persicae* obtained by Davies in North Wales could be applied to Devon and Cornwall, since, if this proved to be the case, the possibilities for the growing of health certificated potatoes for seed purposes could then be explored.

The survey was commenced in 1937 and has been continued each year up to and including 1941. The centres selected for the purpose were, to some extent, chosen as being likely to comply with Davies's findings as to suitable centres for seed production, but a fair proportion was also selected as being likely to yield different results, for purposes of comparison.

The technique employed for the estimation of aphis populations was that of Davies, i.e. a random sample of 100 leaves was examined across the field, thus obtaining an aphis population index figure. The number of *M. persicae* present was counted and they were subdivided into winged, wingless and parasitized individuals. The percentage of leaves infested was also noted. Although no counts were taken of other species, observations were made on the extent to which they were present. A reading of the relative humidity was made at the time of counting, by means of a whirling hygrometer and observations were also made of the direction and force of the wind (Beaufort scale). These observations were of value as they could, to some extent, be linked with regular observations made each day at Seale-Hayne Agricultural College. Notes were also made on the

variety of potato grown, the aspect and height above sea-level of the field and the proximity of winter cruciferous and other crops, which are winter hosts of *M. persicae*. While it was not possible to visit all centres on a number of separate occasions, some of the centres in both counties were visited at intervals, in order to obtain a connected account of the progress of infestations; several centres were visited in this way over a period of several years in order that proper comparisons might be made between one year and another.

The full results obtained during the 5 years of the survey would occupy too much space to be set out in full. A map of Devon and Cornwall (Fig. 1) has, therefore, been prepared which shows the situation of the 107 centres at which counts were made. After each centre is given the height above sea-level; and the highest and lowest counts of *M. persicae* obtained during the 5 years are placed in brackets. Counts include winged, wingless and parasitized aphides. For example, (2–27) indicates a lowest population index figure of 2, and a highest index of 27, during the 5 years' survey. Where only one figure appears in brackets, e.g. (−2), it indicates that only one count was made at this centre during the 5 years. It is not possible in such a map to indicate during which year any particular count was made; a full table of all the results obtained has, therefore, been lodged with the British Museum of Natural History. Owing to the amount of information which has had to be included in the map, the name of the centre and other information is led to the centre on the map by means of a black line. A + sign indicates that results of especial interest were obtained, and that these are dealt with in one of the short tables elsewhere in this paper (Table 3).

Fig. 1 shows that the highest count of *M. persicae* obtained during the 5 years was 609, and this was in a sheltered garden in Newton Abbot on potatoes interplanted with Savoy cabbage. Only a small number of centres yielded counts of over 100 *M. persicae* per 100 leaves, and it is clear that *M. persicae* populations in Devon and Cornwall may be regarded, on the whole, as light. Nevertheless, there clearly exist marked differences between various parts of the two counties in this respect. At only seven centres during the 5 years' survey were total counts of *M. persicae* (including winged, apterous and parasitized individuals) found to exceed 100. Of these one was in a sheltered market garden near Bude, and this was the only centre situated in the area now considered to be the most suitable for seed production.

Distribution of different species of aphides

The following notes on distribution apply to the 5-year period of the survey and not to any individual year.

(*a*) Myzus persicae *Sulz.*

The survey dealt largely with this species, since it is generally considered to be the most important insect vector of potato virus diseases. *M. persicae* was found at fifty-four of the eighty-six centres in Devon and at sixteen of the twenty-one centres in Cornwall; this does not necessarily mean that *M. persicae* was absent at those centres where a count of nil was obtained. This species was found at a great variety of altitudes ranging from sea-level to 1000 ft.

(*b*) Macrosiphum solanifolii *Ashm.* (gei *Koch.*)

M. gei was often plentiful and was found at forty-eight centres in Devon and eleven in Cornwall. It occurred at low and high altitudes and in the north and south of both counties.

(*c*) Aphis rhamni *Boyer*

This species did not occur at as many centres as *M. persicae* or *M. gei*, but produced some of the heaviest populations seen of any species. It was found at twenty-four centres in Devon and at one in Cornwall. It occurred at a great variety of altitudes and in widely separated districts.

(*d*) Myzus solani *Kalt.* (pseudosolani *Theob.*)

Never found in large numbers, but recorded at seventeen centres in Devon and at eight centres in Cornwall.

(*e*) Aphis fabae *Scop.*

Found at twenty-eight centres in Devon and at five centres in Cornwall; in a few cases the populations were fairly large.

(*f*) Myzus ornatus *Laing*

This species, which seems to be exhibiting an increasingly wide range of hosts, was found on potatoes at a small number of centres. Originally described by Laing (1932) as a pest of violets and anemones, it has also been found on strawberries and is of economic importance (Staniland, 1932, 1933). Loughnane, in Ireland (1939), showed that transmissions of potato virus *Y* and leaf roll could be made using *M. ornatus* as a vector. Since this species was found at five centres in Devon and at four centres in Cornwall, live material was sent to Dr K. M. Smith at the Plant Virus Research Station, Cambridge. Dr Smith very kindly carried out transmission experiments, but wrote later to say that all the experiments had given negative results. At most of the centres where *M. ornatus* occurred, the explanation of its occurrence on potatoes was readily found in the presence of other host plants. Two of the centres in Devon were growing anemones and violets, one growing anemones, violets and strawberries, one growing strawberries and violets, and the fourth growing strawberries. At one centre no explanation could be found, though anemones are grown some 4 miles

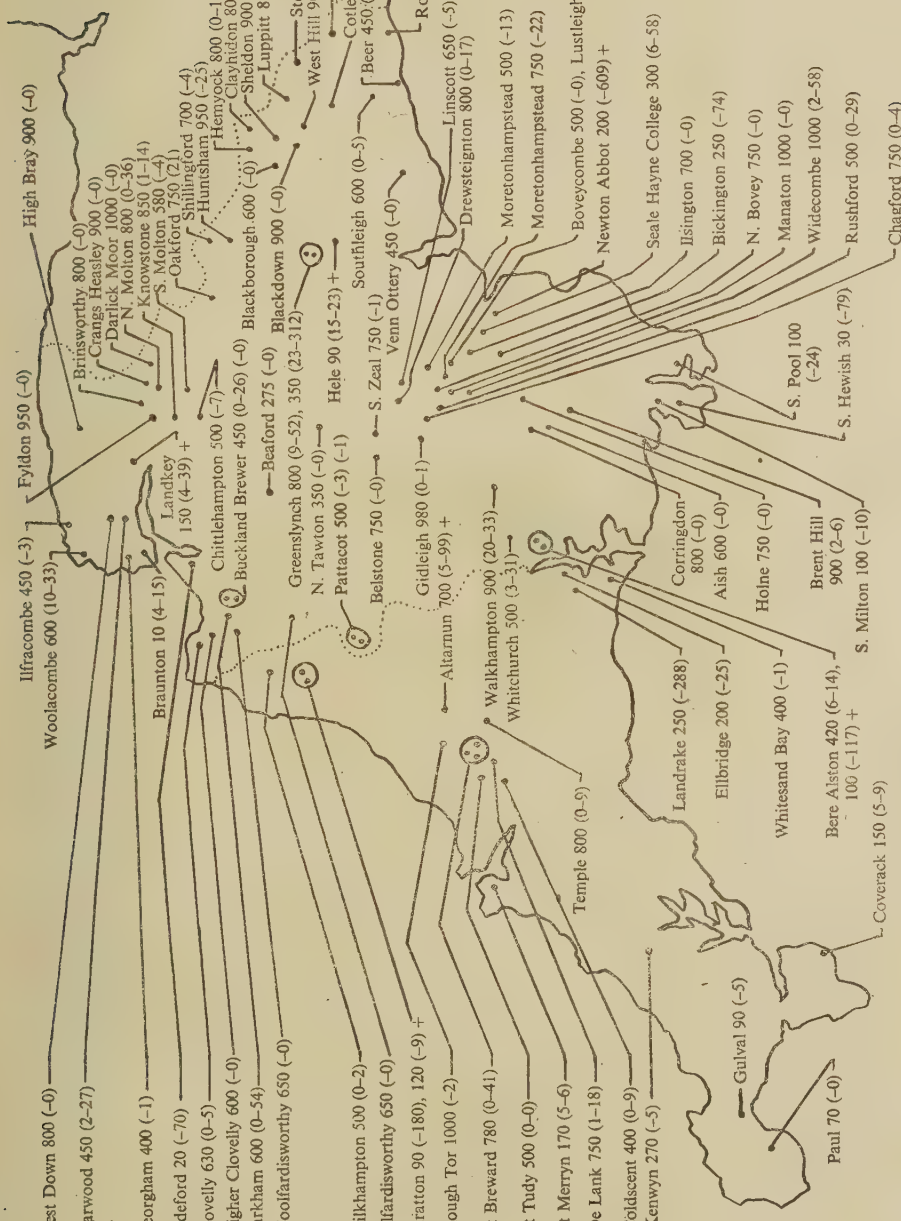

Fig. 1. Distribution of populations of *M. persicae* Sulz. in Devon and Cornwall.

away. Of the four centres in Cornwall, three were growing anemones and violets and the fourth was within a few miles of districts where these crops are grown.

The yearly fluctuations of aphis populations from 1937 to 1941

(*a*) Myzus persicae

The average population index figures for all centres in Devon and Cornwall for the years 1937–41 were as follows:

1937	11·4	1940	0·1
1938	36·9	1941	43·0
1939	43·0		

Table 1

Species	No. of centres at which found: 1940	1937–41
A. rhamni	14	25
M. solani	9	25
M. solanifolii	11	59
A. fabae	1	33
M. persicae	3	70
M. ornatus	0	9

Seasonal behaviour of *Myzus persicae* in Devon and Cornwall

Whilst detailed observations were not made, it can be stated that winged forms of *M. persicae* began to appear in Devon and Cornwall during May in the earlier districts and during June in the later districts. The peak period of infestation was mainly during July, but in some exposed situations the peak period was as late as August. During August and September winged forms again appeared.

Parasitism of *M. persicae* was found to be very variable, but in some instances it was fairly heavy. Table 2 sets out the results obtained in three fields on the same farm in Devon. The fields were at different altitudes with consequent variations in the degree of exposure and humidity. Counts were made on 8 and 30 June and 13 July in all three fields in 1939, which was a year of heavy infestations of *M. persicae*. The figures show the effect of parasites in checking the increase of *M. persicae*. At centre 16, parasitism was fairly heavy and prevented the aphis from increasing to a peak figure in mid-July. At centre 17 parasitism was less than half that at centre 16, and, though it slowed down the rate of increase of *M. persicae* to some extent, there was a high figure of infestation in mid-July. At centre 2, parasitism was initially higher than at either of the other two centres and rose to a greater level; the infestation of *M. persicae* decreased noticeably. The figures illustrate the great variation which may occur in a group of fields close to one another and points to the danger of generalizing in respect of any particular district. Counts made at these centres in other years showed that centre 16 was superior to either centres 17 or 2 for the growing of seed potatoes, as it normally showed lower aphis infestations.

Table 2

Map ref.	Date	Aspect	Variety	*M. persicae* per random sample of 100 leaves: Winged	Apterous	Parasitized: No.	Parasitized: %	Total	% leaves infested
Centre 16 Greenslynch (top field)	8 June	800 ft. S.W. exposed (but sheltered hollow on one side of field)	May Queen	8	43	1	1·9	52	20
	30 June			2	42	3	6·4	47	27
	13 July			2	12	11	44·0	25	21
Centre 17 Greenslynch (middle field)	8 June	350 ft. S.W. sloping	Arran Pilot	4	141	3	2·0	148	49
	30 June			2	259	8	2·9	269	80
	13 July			14	242	56	17·9	312	78
Centre 2 Hele (bottom field)	8 June	90 ft. flat	May Queen	7	95	5	4·6	107	41
	30 June			2	91	20	16·2	123	57
	13 July			1	33	32	48·4	66	41

(*b*) *Other species*

During 1940, which was a year of light infestation by *M. persicae*, the numbers of other species also dropped considerably, and a note as to the number of centres at which the various species were found in 1940, as compared with the figures already given for the whole of the 5 years, indicates the extent to which such reduction took place (see Table 1). The only three centres at which *M. persicae* was found in 1940 were in districts which have consistently shown low population index figures; no explanation of this can be offered. *Aphis rhamni* was least affected in 1940 as regards the number of centres at which it was found; indeed the heaviest populations of this species were observed in this year.

Relationship of proximity of winter hosts to aphis population

The survey in Devon and Cornwall emphasized the importance of the proximity of the various winter

hosts of *M. persicae* in relation to aphis populations. *M. persicae* was found during the winter on swedes or broccoli, Brussels sprouts, cabbage, kale and savoys. It was most common on savoys and Brussels sprouts, of which the former was by far the most favoured host. Both Davies (1939) and Jacob (1941) found this to be the case and ascribed the preference of the aphis for savoys to the shelter afforded by the crinkly leaves. The insects were always found as apterae and no eggs were seen.

Other winter hosts of *M. persicae* of special importance in Devon and Cornwall are anemones and violets, both of which are grown commercially for flower production in various districts. Infestations on these plants were observed in every year, and were frequently heavy. In the milder districts both plants were heavily infested for a great part of the winter and became smothered with the aphides in March and April. Close proximity of winter host plants to potatoes was found to result in higher populations of *M. persicae* than on potatoes which were isolated. These differences were markedly more apparent in years of heavier infestations than when populations were light. In those parts of the counties where populations are normally light the proximity of winter hosts did not appear to increase populations to such an extent as in other areas. Table 3 gives instances of the relations between winter host plants and populations of *M. persicae* on potatoes.

Relationship of meteorological conditions to populations of *Myzus persicae*

Davies's findings on the relationship of humidity, temperature and exposure to wind, appear to hold for the counties of Devon and Cornwall. The population index figures were generally low in those districts in which humidities were frequently high, and in which there was exposure to winds. Regular readings of relative humidities, rainfall, temperature and direction and force of the wind were available (made at 9.0 a.m. each morning) up to the outbreak of war, from Seale-Hayne Agricultural College, which lies in a district of relatively high aphis infestation. Comparison of these records with observations and readings made at the time of population counts showed that areas with low population index figures were generally much more humid and more exposed to winds than were the areas from which high counts were obtained. Whilst a far greater volume of meteorological records would be required to enable exact comparisons to be made between the years 1937–41 as regards varying aphis populations, sufficient information is to hand to explain, in part, the variations found.

Exposure to wind is considered specially important. Records at Seale-Hayne Agricultural College were used for the drawing of wind roses, in order to give a picture of direction of winds for the various months and at the same time to indicate the sum of the Beaufort scale of wind forces from each direction. Wind forces of 2 and over were taken as being of sufficient force to affect aphis migration. Figs. 2–5 are of wind roses showing direction and force of winds for May and June of the years 1937 and 1939. 1937 was a year of light infestation of *M. persicae*, and there was a markedly greater amount of strong winds from the south-west and north-west, particularly in June. As a contrast, Figs. 4 and 5 show that in 1939, which was a year of heavy aphis infestation, there was a far greater proportion of very dry north-east winds and less of the south-west and north-west winds. Strong winds usually come from westerly quarters, easterly winds usually being light. A total of wind-force figures (of force 2 and over) from all quarters for April–May in each year was found to bear a relation to the degree of infestation prevalent in those years.

Table 4 sets out the meteorological conditions for the 5 years of the survey, the figures given being for the important months of April, May and June in each year, during which infestations of *M. persicae* take place. The table also shows that there is a definite correlation between high humidity, high rainfall, strong winds and low aphis populations. The populations of *M. persicae* in 1940 were exceptionally low, and a factor, additional to those stated above, was the unusually severe winter of 1939–40. Though the winters of 1940 and 1941 were also more severe than normal, there was little or no damage to winter Brassicae, whereas in the winter of 1939–40, these Brassicae suffered severe damage, many being killed, as the result of a 'silver thaw'.

Altitude in relation to populations of *Myzus persicae*

The results obtained during the 5 years' survey show that altitude has no direct relationship to populations of *M. persicae*, in the sense that the greater altitudes were associated with lower populations. Many low populations were found at low altitudes, approaching sea-level, these centres being in situations where humidities were high and where there was much exposure to winds, particularly moist westerly winds; centres nearby, however, often yielded lower populations when they were situated at a greater altitude, by reason of increased exposure to wind. This was noticeably the case in districts such as the eastern portion of Devon, where normally higher populations obtain than in the north and west of the counties. Centres in such districts when at high altitudes gave low populations due to increased exposure and higher humidities.

Conditions for production of seed potatoes in Devon and Cornwall

As a result of investigations by other workers and of the present survey, the conditions which are of importance for the production of first-grade seed

TABLE 3

Centre no.	District	Date	Altitude and aspect	Variety	*M. persicae* per 100 leaves: Winged	Apterous	Parasitized	Total	% leaves infested	Remarks
6 (Devon)	Landkey	6 June 1937	150 ft., S., sheltered	Sharpe's Express	11	20	8	39	27	1937 was a year of fairly light infestation. This centre gave one of the higher population index figures for Devon in 1937. The district as a whole is one of low infestation. The field adjoined a market garden growing vegetables, fruit, anemones and violets
24 (Devon)	Bere Alston	14 June 1938	420 ft., very exposed to W.	Epicure	4	1	1	6	6	Some Brussels sprouts and broccoli grown for seed and anemones. Allotments near
25 (Devon)	,,	14 June 1938	100 ft., sheltered, near river and facing almost S., about 1 mile from centre 24	Arran Pilot	1	115	1	117	39	Much broccoli grown on farm: also anemones. Additional exposure at centre 24 kept population low despite presence of winter hosts at both centres. Warmer conditions at centre 25 also favoured increase
7 (Cornwall)	Stratton	2 June 1937	90 ft., warm and sheltered	Midlothian Early	160	20	—	180	82	Sheltered market garden with winter Brassicae
8 (Cornwall)	,,	2 June 1937	120 ft., fairly exposed	King Edward	2	7	—	9	9	A field lying two fields removed from market garden of centre 7 above. Winter Brassicae also growing in field. 1937 was a year of fairly light infestation
6 (Cornwall)	Altarnun	2 June 1937	700 ft., fairly exposed	Sharpe's Express	2	—	—	2	2	A school garden high and fairly exposed on Bodmin Moor, which is a district of usually low infestation, 1937 being a year of fairly light infestation
Do.	,,	29 June 1937	,,	,,	—	6	—	6	4	A second count made on the same potatoes
Do.	,,	20 June 1938	,,	,,	17	81	1	99	49	Count made on the same variety of potatoes in the same school garden in a year of medium infestation (1938)

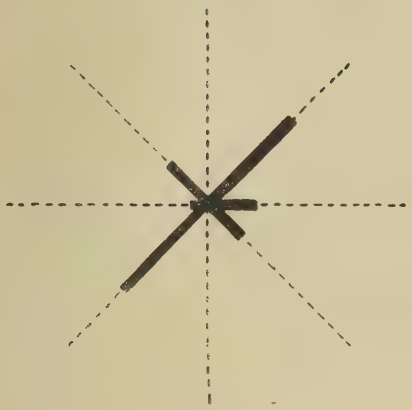

Fig. 2. May 1937.

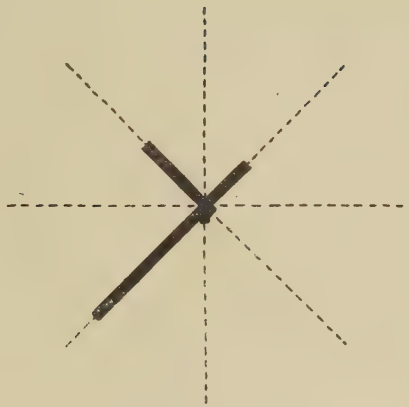

Fig. 3. June 1937.

(Infestations of *M. persicae* were light in 1937.)

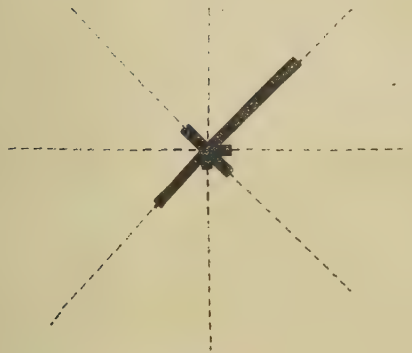

Fig. 4. May 1939.

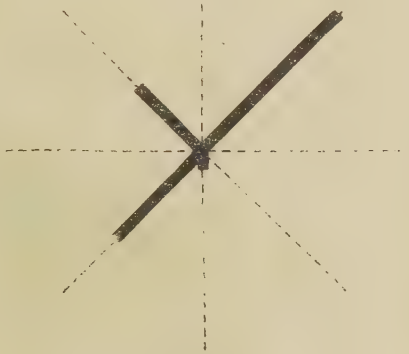

Fig. 5. June 1939.

(Infestations of *M. persicae* were heavy in 1939.)

Figs. 2–5. Wind roses showing direction and force of winds for May and June of the years 1937 and 1939.

TABLE 4

Month	1937	1938	1939	1940	1941
			Relative humidity		
Apr.	83·1	66·9	77·4	80·0	71·0
May	81·7	72·9	72·5	75·0	67·0
June	72·5	72·7	88·7	68·0	81·0
			No. of days on which humidity was 70% or over		
Apr.	25	11	21	23	13
May	26	18	21	23	14
June	14	23	11	13	27
			Rainfall		
Apr.	3·61	0·08	3·47	3·96	1·08
May	2·98	2·72	0·50	1·53	2·43
June	1·22	1·21	3·35	0·89	2·72
			No. of days on which winds were of force 2 and over		
Apr.	25	11	21	28	9
May	25	18	21	31	8
June	21	23	11	29	6
Average populations index figure for *M. persicae*	11·4	36·9	43·0	0·1	43·0
Order of populations of *M. persicae* from small to large	2	3	4	1	4

potatoes in Devon and Cornwall may be set out as follows:

(1) The potatoes should be grown in districts which give normally low population index figures for *M. persicae*: such figures should not exceed 100. This means that they should be grown in districts in which humidities are frequently high and in which there is much exposure to winds, particularly moist westerly winds.

(2) The actual fields employed for the purpose should have the characteristics of the district in which they are situated. For final selection, each farm or holding, though it may be situated in a suitable district, must be judged on its own merits as a seed producing centre.

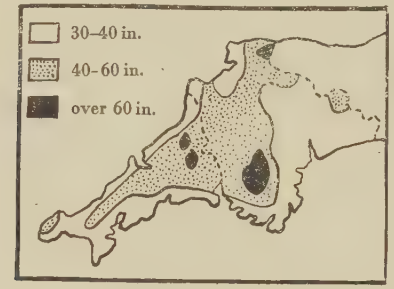

Fig. 6. Rainfall map of Devon and Cornwall.

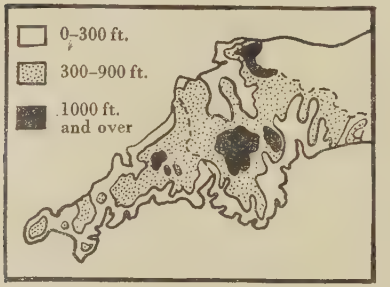

Fig. 7. Altitude map of Devon and Cornwall.

(3) The crops must be isolated from any area growing cruciferous plants, especially winter Brassicae, on any large scale, and also from anemones and violets. In practice, some of these crops can be found in most districts, but the conditions would be satisfied if seed growing is not carried out near market gardens, towns with many gardens or allotments, or districts given over to any extent to broccoli.

In addition to the above conditions it is of importance that the district should be one in which the farms are free from potato eelworm; also the soil should be reasonably suitable for the potato crop, and a 'potato-minded' farmer who will take the necessary interest and trouble with the crop in order to produce a sound and healthy crop of seed, is an important element in success.

Areas suitable for the production of first-grade seed in Devon and Cornwall

Taking into consideration the results of the aphis survey and the conditions for successful seed-potato production which have been set out, the approximate distribution of suitable areas can be delineated. Fig. 9 shows the situation of those districts suitable for the production of TSA seed: there are four areas, A, B, C and D. Conditions 1, 3 and 5 in the previous section, taken into consideration with the results of

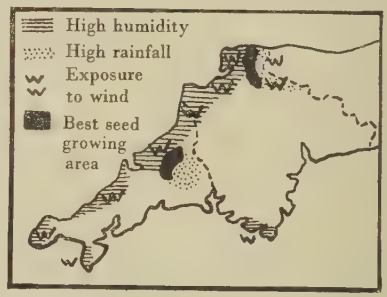

Fig. 8. Map showing areas of high humidity, high rainfall, exposure to winds and the theoretically most suitable areas for seed-potato production.

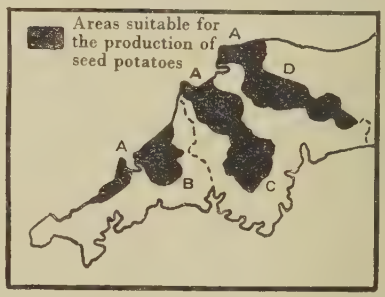

Fig. 9. Approximate situation of areas in Devon and Cornwall suitable for seed-potato production.

counts made in the course of the survey, led to the rejection of west Cornwall on account of the frequent cultivation of broccoli, anemones, violets and various vegetable crops, and also on account of certain areas affected with potato eelworm, despite the fact that some low aphis counts had been obtained in parts of that area. South and east Cornwall were rejected for somewhat similar reasons, as also was south Devon and the greater part of south-east Devon.

The map shows that the four suitable areas are as follows:

Cornwall:

A. A strip of country along the Atlantic coast. Much of this area is well below 400 ft. above sea-

level, though in parts it is as high as 700 ft. It is, however, a very exposed and humid district.

B. This area includes Bodmin Moor and much of the country lies at 800 ft. above sea-level and the Moor itself is 1000 ft. and over. The coastal region of this area being higher than in area A is even more exposed.

C. This area includes the large moorland area of Dartmoor, together with a large area of high, exposed land between Dartmoor and the Atlantic coast.

Dartmoor lies between 800 and 1600 ft. above sea-level and parts rise to a height of over 2000 ft. The area between Dartmoor and the Atlantic coast lies mainly between the 400 and 600 ft. contours, and this high land extends within a few miles of the coast. Part of this area lies in north Cornwall.

D. This area includes that portion of Exmoor which lies within the Devon border.* Exmoor, in Devon, rises to a height of over 1500 ft. above sea-level, and the country between Exmoor and the northern coast all lies between 400 and 800 ft., with various small areas which are nearly 900 ft. Land of similar altitude lies south-west of Exmoor and continues in a long strip running in a south-easterly direction along the Devon-Somerset border. This strip contains much land between 700 and 800 ft. above sea-level and a number of moorland areas such as the Blackdown Hills and Blackdown, which are at nearly 1000 ft.

It is of interest to consider the counties of Devon and Cornwall as to their suitability for seed-potato production from a theoretical standpoint, bearing in mind that the conditions are low populations of aphis as the result of high humidities and exposure to wind. Fig. 6 shows that a large portion of Devon has an annual rainfall of from 40 to 60 in., including an area in the region of the Blackdown Hills, with areas in Bodmin Moor, Dartmoor and Exmoor, where the annual rainfall is considerably in excess of 60 in. High rainfall is linked with high humidities. Altitudes are shown approximately in Fig. 7, and it is seen a large portion of the two counties lies between 300 and 900 ft., with areas in Bodmin Moor, Dartmoor and Exmoor of over 1000 ft. Altitude is linked with rainfall and humidity. Fig. 8 shows the areas of high humidity lying along the west-facing coasts of the two counties, and also the districts in which there is exceptional exposure to winds. Particularly suitable areas for seed production would be expected in those districts where the humid coastal regions adjoin high, exposed land with a high rainfall. From these considerations the west sides of Bodmin Moor and Exmoor should, theoretically, be areas of especial suitability for seed-potato production. The aphis survey and subsequent observations on stocks of potatoes grown for seed support this conclusion, though also showing that other areas not quite so exposed are equally suitable for seed production.

* The greater part of Exmoor lies in Somerset, and much of it is probably suitable for seed-potato production. The survey has, however, been confined to the counties of Devon and Cornwall.

The formation of a 'Seed Potato Growers' Association' in Devon and Cornwall

After the survey of potato aphides in Devon and Cornwall had been carried on for 3 years, it was evident that these counties contained suitable areas for seed production. The outbreak of war had rendered the production of healthy seed potatoes of first importance, and it was clear that everything was to be gained by applying the results of the survey as soon as possible. A start was made in October 1939, and during this and succeeding months, a large number of farmers in suitable areas were visited, arrangements were made for the growing of seed potatoes and the interest of farmers in the formation of a 'Seed Potato Growers' Association' was aroused. Rules for the conduct of such an Association were framed and received the approval of the Ministry of Agriculture. Foundation stocks of a number of varieties known to be in demand were obtained from Eire.

During the first season the Association consisted of fifty-four members, who grew a total of 152 acres for seed. The stocks were carefully rogued, both for virus diseases and for trueness to variety and an excellent series of stocks resulted. During the following season, 1941, the membership and the acreage of potatoes grown increased, and inspection of the stocks during the growing season showed that the health of the stocks had been maintained at a high level, practically all the stocks reaching TSA standard. No complaints were received during the 2 years' sales of seed. Mr R. Wellington, of Bradninch, Devon, was in great part responsible for the organization of the Association, for the purchase and distribution of stocks, and for the sales of seed.

Acknowledgement is due to Mr J. C. F. Fryer, Director of the Ministry of Agriculture Plant Pathological Laboratories, Harpenden, Herts, for much help and valuable criticism during the course of the work, and to Mr A. Beaumont, who has read through the manuscript and given much help with the meteorological data. Mr J. F. Perkins was of great assistance during the years 1940 and 1941 in the making of aphis counts.

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(*Received* 4 *May* 1942)

The mode of action of pyrethrum on the cockroach, *Periplaneta americana* L.

BY D. N. ROY, S. M. GHOSH AND R. N. CHOPRA
School of Tropical Medicine, Calcutta, India

Pyrethrin, the active principle of pyrethrum powder, is insoluble in water but is soluble in the body fluid of the cockroach. It has a selective action on nerve ganglia, and the destruction of their cells is responsible for the death of the insect. Whereas pyrethrum, when used either in the powdered form or in a fluid state mixed with kerosene and introduced directly into the body cavity reaches the ganglion with the circulation, its mode of action when it acts through the spiracles is different. Kerosene-pyrethrum mixture when introduced into the tracheal trunks through the spiracles is quickly diffused into the haemocoele. When the dry powder is inserted into the trachea, its mode of action is analogous to a fluid preparation. As the conversion of pyrethrum from a dry into a fluid state is alone possible in the interior of the trachea, the natural conclusion is that a fluid analogous to the body fluid is present in the same situation. As soon as the pyrethrin is dissolved, it is quickly diffused into the haemocoele and thus reaches the nearest ganglion.

Pyrethrum, a deadly neuro-muscular poison, when introduced into the body of an insect, has a tendency at first to cause irritation, as is evident from the fact that soon after injection the insect, when confined in a closed space, walks round and round at a rapid pace. The gait soon becomes staggering and thereafter marked weakness of the legs is noticed. This eventually proceeds to paralysis of the muscles. Such action may be either extremely rapid or slow, and may be partial or complete, according to the quantity of pyrethrin injected and the nature of the insect on which such experiments are performed. When the weakness or paralysis is partial, recovery takes place. Pyrethrin, the active constituent of pyrethrum, is soluble in cold water to a negligible extent (Chopra *et al.* 1940), but is readily soluble in kerosene, petroleum ether and other solvents, and to some degree in hot water (Roy *et al.* 1941). Pyrethrin is insoluble in normal salt solution and in solutions of urea, glucose and lactic acid.

ACTION OF PYRETHRUM ON THE COCKROACH

In our experiments, which were all performed on the cockroach, *Periplaneta americana* L., we generally used a mixture of kerosene and pyrethrum (1–0·0025), so that 0·01 c.c. dose represents 0·25 mg. of pyrethrin. Kerosene is toxic to the cockroach but its action is extremely slow. The progressive nature of the weakening or pseudo-paralysis of the legs can best be studied by injecting a small quantity of a

mixture of pyrethrum and kerosene either into the abdomen or one of the spiracles. How the paralysis starts in different legs according to the part of the body selected for the introduction of the toxic fluid is shown in Table 1.

The results obtained may suggest that pyrethrin circulating in the haemocoele fluid acts directly on the peripheral motor nerves thereby inhibiting their activities, but this is not the case. If at the stage when the activity of the legs has been markedly inhibited by pyrethrin, a hind-leg is removed by cutting through the coxa, alternate contractions and relaxations of the leg will continue for a long time, in some instances over ½ hr. In a normal insect movements of an excised leg, if they occur at all, are feeble and of short duration (not more than three to four feeble contractions may be noticed). This proves that pyrethrin has no inhibitory action on the nerves that supply the legs. On the other hand, injection of a minute quantity of the toxic fluid into the substance of the third thoracic ganglion, or application of the powder on its outer surface, after it has been exposed in a living insect, is followed by immediate paralysis of the third pair of legs, which remains entirely localized and does not spread to the other legs.

When the continuity of the abdominal nerve cord has been severed by complete excision with a sharp-edged needle somewhere in the upper part of the abdomen, and thereafter the fluid is injected into the posterior part of the abdominal cavity, paralysis of the legs occurs in the same way as when the injection has been made into a healthy insect. This suggests that the connective fibres of the central nerve chain play an insignificant part in the action of pyrethrum.

We have proved that pyrethrum produces no effect on a cockroach when it is swallowed with its food either in a dry or in a fluid state.

How pyrethrin circulates in the body

It is apparent that pyrethrin when introduced into the abdominal cavity is carried to different ganglia with the body fluid. But in absence of any known direct connexion between the spiracle and nerve ganglia, it is hard to explain how pyrethrin acts when it is injected through the spiracle.

Kerosene, coloured red with Sudan III, when injected into a large thoracic tracheal trunk through the spiracular opening, can be detected not only in many of the large and smaller tracheae on both sides but in the finer branches also. It seems that it can also penetrate into some of the tracheoles. The main feature noticed in this connexion is the wide and diffuse staining of the loose tissue of the legs and thoracic muscles surrounding the tracheal trunk into which the injection has been given, and this is only possible on account of diffusion of the injected fluid through the tracheal wall. Whether or not kerosene can reach the body fluid along the tracheoles is doubtful, as the coloured fluid cannot be detected either in the tissue cells of the stomach, for example, or in the body tissue immediately surrounding the tracheoles.

Presence of fluid in the tracheae

Evidence of moisture in the tracheal trunks is afforded by gently blowing finely powdered pyrethrum through either of the two thoracic or the first abdominal spiracles. This can be effected under the binocular microscope with the help of a finely pointed capillary pipette, the end being inserted in the spiracular chamber. After the end of the pipette has been inserted in the trachea, some teased cotton-wool is placed around the spiracle so that if by chance any powder has escaped outside, there is less possibility of its coming in contact with the neighbouring spiracles. When pyrethrum in the powdered state is thus inserted in the large tracheal trunks in the thorax, it produces the same characteristic effect on the insect as is noticed when kerosene-pyrethrum mixture is used, and the paralysis follows the same order according to whether the powder has been blown into the first or the second thoracic spiracle. Control experiments with chalk or dust are without any definite results; therefore the action is not one of mechanical blocking.

In explaining its mode of action when the dry powder is blown into the tracheae through the spiracular opening, it is necessary to postulate a solution of the pyrethrin; also when the dry powder is introduced into the body cavity through the abdominal intersegmental membrane, paralysis of the legs ensues. That the body fluid is capable of dissolving pyrethrin can also be demonstrated by withdrawing from a large number of insects the haemocoele fluid with which a minute quantity of pyrethrum has been mixed. This, after dilution with normal salt solution, is filtered and the filtrate is injected either into the body cavity or through the spiracles of other insects. Results identical with kerosene-pyrethrum will be noticed.

Pyrethrum owes its action entirely to pyrethrin which is insoluble in water and, therefore, before it can produce its characteristic effect on the ganglion, on which alone it acts, it must be converted into a solution. We have to demonstrate where this conversion can take place. The probability of the powder being able to pass through the tracheal wall is extremely remote. Neither can it, when blown in, be carried to such a distance as the tracheoles, which, according to Wigglesworth (1930, 1931), are filled with fluid. The extensive intercommunication of tracheae also does not support such mode of action (vide Table 1). The only place, therefore, where the change from the solid to the liquid form can occur must be in the interior of the tracheae. This suggests that normally the tracheal trunks, both large and

TABLE 1. *Effect of pyrethrum on the cockroach,* Periplaneta americana *L.*

Form in which pyrethrum was used	Quantity introduced	Method followed	Site of introduction	Progress of pseudo-paralysis or marked weakening	Interval between time of injection and onset of paralysis	Nerve supply	Remarks
Mixture of pyrethrum in kerosene	0·01 c.c. (0·25 mg. pyrethrin)	Injection	Left fore-coxa	Left fore-leg Right fore-leg Left mid-leg Right mid-leg Left hind-leg Right hind-leg	15 sec. 1 min. 15 sec. 3 min. 5 sec. 4 min. 30 sec. 6 min. 5 sec. 7 min. 10 sec.	From 1st thoracic ganglion	—
Do.	Do.	Do.	Left hind-coxa	Left hind-leg Right hind-leg Left mid-leg Right mid-leg Left fore-leg Right fore-leg	15 sec. 1 min. 10 sec. 3 min. 20 sec. 4 min. 30 sec. 10 min. 11 min. 15 sec.	From 3rd thoracic ganglion	—
Do.	A minute quantity	Injection with a blunt-pointed fine needle	Left anterior thoracic spiracle	Same as occurs when the injection has been given in the left fore-coxa	Both front legs almost immediately	From 2nd thoracic ganglion	—
Do.	Do.	Do.	Left posterior thoracic spiracle	Same as occurs when the injection has been given in the left hind-coxa	Both hind-legs almost immediately	From 3rd thoracic ganglion and probably also from 2nd thoracic ganglion	—
Do.	Do.	Do.	Left first abdominal spiracle	Same as occurs when the injection has been given in the posterior thoracic spiracle	Both hind-legs almost immediately	From 3rd thoracic ganglion	—

Do.	0·01 c.c.	Injection.	Posterior part of the abdominal cavity in the left of the middle line	Left hind-leg Right hind-leg Left mid-leg Left front leg Right front legs	2 min. 15 sec. 3 min. 25 sec. 4 min. 20 sec. 6 min. 30 sec. 14 min., 15 min. 20 sec.	—	—
Finely powdered pyrethrum	A 'minute' quantity	By blowing through a capillary pipette provided with a rubber teat at the other end	Left anterior thoracic spiracle	Marked in the left front leg, feeble in the right front leg, and feebler in the left middle leg	Acts almost immediately on the left front leg	—	Recovery generally takes place
Do.	Do.	Do.	Left posterior thoracic spiracle	Starts in the left hind-leg	Acts almost immediately on the left hind-leg	—	Do.
Body fluid of the insect with which pyrethrum has been mixed: the mixture is thereafter diluted with salt solution and filtered	—	Injection	Left posterior thoracic spiracle	Left hind-leg	—	—	Do.
1% gentian violet	0·01 c.c.	Injection	Anterior and posterior thoracic spiracles	No sign of weakness of any leg	—	—	The presence of the dye can be detected in the tracheae of the same and also of the other side in the head and thoracic regions. The tracheoles are never found injected. The loose tissue around the tracheal trunk in which the dye has been injected is deeply infiltrated with the stain

small, contain a certain amount of moisture which, like the haemocoele fluid, has the power of dissolving pyrethrin.

The action of pyrethrum on nerve endings

It might be thought that the quick action of pyrethrum manifest in the legs when it acts through the spiracle is because the impulse originating in the sensory end-plates may be directly conducted along the sensory nerves to the ganglion which innervates the spiracle. But the nerve endings of the spiracles can easily be paralysed with 1 % solution of cocaine hydrochloride and, as pyrethrum still acts under such conditions, it clearly indicates that the sensory nerves play no role in its action. The campaniform sensilla, which are so widely distributed over the body of the cockroach (Pringle, 1938), though sensitive to mechanical stimuli, do not respond when pyrethrum either in a fluid or powdered form is applied over them. When kerosene-pyrethrum mixture is injected into the substance of the exposed third thoracic ganglion or the powder is applied on the surface, only the hind-legs are paralysed. As the other legs are not affected, the action is arrested in the ganglion. In this way only one-half of a ganglion is attacked, leading to paralysis of the leg of that side only.

Theory of diffusion through tracheae

If pyrethrum, when introduced through the spiracle, is converted into a fluid in the tracheae, how can it travel from the interior of the tracheae to the thoracic ganglion? When the dry powder or kerosene-pyrethrum mixture is introduced through the first thoracic spiracle the front leg of the same side is first affected, and, when it enters through the second thoracic spiracle, weakening or paralysis of the hind-leg of that side is first noticed. It appears as if there is a direct connexion between the spiracle and the corresponding thoracic ganglion.

The possibility of the toxic material reaching the body cavity along tracheoles can be ruled out. The only direct connexion that can be established is through its action on sensory nerve endings in the spiracles. It has already been shown that pyrethrum cannot act on sensory or on motor nerve fibres. Therefore, the only way it can act is by diffusion in a fluid state from the interior of the tracheae to the haemocoele through the tracheal wall from where it is carried with the body fluid to the ganglion.

Explanation of the selective action of pyrethrum on a particular ganglion according to which spiracle is selected for injection

Assuming that pyrethrin, being dissolved in the tracheal moisture diffuses out into the body fluid, we have yet to demonstrate why the action should start in different legs according to which spiracle is selected for injection. This can better be explained by referring to the following observations. When 1 % gentian violet is injected through the thoracic or abdominal spiracles, care being taken that the dye does not escape into the loose space underlying the prothoracic tergal plate, different results are obtained (vide Table 1). On dissection, the tissue immediately surrounding the large tracheal trunks close to the particular spiracle which has been selected for injection will be found deeply infiltrated with the dye. The tracheoles are never found injected. When kerosene, coloured red with Sudan III, is used in place of gentian violet, similar phenomena are noticed. These indicate that the nearness of a particular thoracic ganglion to the loose space surrounding the large tracheal trunk, in which the pyrethrum, either in a fluid or powdered state, has been inserted, accounts for the start of paralysis in a particular leg when the injection has been made in a certain spiracle.

Discussion

Pyrethrin, the active principle of pyrethrum powder, is insoluble in ordinary water, in a saturated watery solution of carbon dioxide or in 3 % lactic acid. It is, however, soluble in such substances as kerosene or petroleum ether; also in the haemocoele fluid of the cockroach. When it is introduced into the body cavity of the insect, the progressive nature of paralysis of its legs indicates that the pyrethrin is carried with the circulation of the body fluid. Experimental results clearly indicate that pyrethrin has a selective action on ganglion cells which may even be localized to one-half of a ganglion, causing paralysis of the leg of one side and not of the other. In certain respects this confirms the findings of Wilcoxon & Hartzell (1933) and of Hartzell (1934), that destruction of nerve tissue in the ganglia is a primary factor in causing death of an insect killed by pyrethrum.

As pyrethrum, when used either in the form of a dry powder or kerosene-pyrethrum mixture, acts equally effectively through the spiracles, it must be realized that the principles involved in such cases are different. A 'kerosene mixture' may act when introduced into the 'spiracle' in one of the following ways: (1) it may be carried along the large and small tracheal trunks and thereafter possibly along tracheoles into the body cavity; (2) as the tracheoles may have semi-permeable walls, the kerosene-pyrethrin may pass into the body fluid through tracheoles; (3) the fluid may be diffused quickly through the tracheal wall into the body cavity.

In the first two conditions the action is bound to be considerably slower than when the toxic fluid is introduced directly into the abdominal cavity. Furthermore, under such conditions the paralysis would be more diffuse and would not be manifest in only one leg as when the fluid is injected into any particular spiracle.

The diffusion theory of the modus operandi of

fluid preparation of pyrethrum has been based on experimental evidence. Any theory advanced to explain the mode of action of dry pyrethrum when inserted in the spiracle must be based on the following findings: (1) it acts extremely quickly; (2) its action is first manifest in the same leg as would occur when the kerosene preparation is used; (3) pyrethrin, which is insoluble in water, carbonic and lactic acid, is at least partially soluble in the body fluid. The same diffusion theory, therefore, holds good in the case of pyrethrum when used in a powdered form through the spiracle, and its action is analogous to that of a fluid preparation. As conversion of solid pyrethrum into a liquid state is a prerequisite condition for its penetration into the body cavity through the tracheal wall, the presence of a certain amount of moisture in the lumen of the trachea can reasonably be assumed.

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(*Received* 3 *March* 1942)

Materials for a study in animal competition. The fauna of the sewage bacteria beds. Part II

By Ll. Lloyd, D.Sc.
University of Leeds

An assessment of the numbers of flies emerging from a bacteria bed has been almost continuous through nearly 8 years, and an attempt is made to explain the reasons for the specific fluctuations. These are found to depend on weather influences reacting on *Metriocnemus longitarsus* and the worm *Lumbricillus lineatus*, two forms which dominate the upper region of the bed, the former being potentially predaceous.

The annual cycles of *M. longitarsus* are so timed that there is a major flight in May establishing its offspring in June. If June is cold and wet this form is successful and a barrage of larvae is formed in which much oviposition of the other species takes place. The eggs are largely destroyed in the barrage. Hot dry Junes are unfavourable to establishment and the barrage is thinned, or it may be dispersed by subsequent dry conditions. *M. hirticollis* has a major flight about a month later and so is dependent for summer establishment on a dearth or dispersal of *M. longitarsus* larvae. *Spaniotoma minima* is similarly affected, but the effect is less lasting because its cycles are rapid and it has the capacity for recovery in the cooler months which leads to a reasonable flight in May. The thermal requirements of *M. hirticollis* do not allow of this except in a very mild winter, and consequently it cannot recover from collapse until a season recurs in which hot dry conditions cause failure or dispersal of the barrage. The distribution of weather was such that gradations with a 4-year interval were set up in the annual incidence of these flies.

When the larvae of *S. minima* (and possibly, but not distinctively, those of *M. hirticollis*) are reduced by abundance of *M. longitarsus* larvae, pressure in the depths of the bed is relieved of a proportion of forms also potentially predaceous. By this the psychodids, which probably always oviposit to some extent in the depths, are benefited and are able to extend their seasons of abundance later in the year than usual.

The numbers of *S. minima* and *M. hirticollis* emerging in the early part of the year are largely determined by their autumn abundance, so the fluctuations of the whole year are referable to interspecific competition regulated by summer weather. This is not so for the other common flies which are markedly influenced by events in winter as well as in summer. There is a tendency for *Psychoda alternata* to be more abundant after a warm winter because its physiological requirements are a paramount factor, but some evidence is found that extra pressure of the other larvae may reduce it then as they do later in the year. Conversely the numbers of *M. longitarsus*, and still more of *P. severini*,

tend to be less after a warm winter in spite of the fact that they are essentially winter-breeding insects. This is attributed to the intervention of *L. lineatus* in competition because the worm breeds mainly in the winter months and its thermal requirements are such that a rise from 7 to 10° C. (about the limits of cold and warm in the beds in winter) causes a great acceleration in its cycle, while cold conditions drive it down from the surface *Phormidium* which it almost completely destroys in spring. Therefore it is suggested that the time of sloughing will be earlier or later in spring according to the winter-bed temperature. This hastens or retards the period of deprivation for the larvae and so regulates distinctively the prosperity of the flies which breed briskly in winter and early spring. As assessments of *L. lineatus* were not made throughout the period these findings are theoretical. It is thus shown that the annual fluctuations of these forms are partly dependent on weather conditions and partly dependent on interspecific competition regulated by the weather. In several cases competition is shown to be the more potent direct influence.

This paper may be regarded as a continuation of that by Lloyd *et al.* (1940) in which interspecific competition was proved to exist amongst the dominant insects of bacteria beds. This is effected partly by deprivation in the over-dense population, and partly by interference including direct attack. It is now proposed to examine the reasons for fluctuation in annual abundance of the various species, to discover the influence of varying climatic conditions, and whether competition has any distinct effect on such fluctuation.

The organisms involved comprise two species of *Metriocnemus* so closely allied that the males seem indistinguishable, *M. longitarsus* Goet. and *M. hirticollis* Staeg., two species of *Spaniotoma*, *S. minima* Mg., very abundant, and *S. perennis* Mg., usually scarce. These are Chironomidae. There are two species of Psychodidae, *P. severini* Tonn., parthenogenetic, and *P. alternata* Say. The enchytraeid worm, *Lumbricillus lineatus* Mull., is also much involved.

Almost continuous trapping of the insects emerging on the beds of the Knostrop (Leeds) Sewage Works extended from the end of 1933 to August 1941 and was on a uniform plan. Two traps were in use for 3 years and later three traps were in action. Larva trapping by three small surface tins was carried on for 3 years. All trapping data given are averages of the two or three traps taken over monthly periods. These are arranged in Table 1 for flies and Table 2 for larvae and pupae. For the flies the average output for each month over the 7 or 8 years is given with the standard error of the mean. Where the index for the month differs significantly from the mean (three times the standard error) it is in heavy type for high and in italics for low value. Where two successive months differ from the mean by only twice the standard error these also are taken to have significance. July was missed twice, and in the tables an intermediate value is supplied except for *P. severini* which is usually scarce in July. These interpolated values are not used in the averages, so those for July depend on 6 years. They are used in calculating correlation coefficients which would have been impracticable without them.

The key to the whole problem depends on some zoning of the organisms in the bed. The larva of *S. minima*, a grazer with a tendency to predacity, is distributed through the depths without a known preferred zone. *M. longitarsus* and *L. lineatus* dominate the top foot of the bed but also occur more deeply. The latter does not intervene in the competition except as a deprivative force and by interference through loosening film and organisms. *M. longitarsus* larva, essentially a grazer, readily becomes predaceous. *M. hirticollis* larva has similar habits except that it does not climb so persistently in the beds. At Knostrop the larvae of *P. severini* and *P. alternata* are much less dense near the surface than deeper in the bed, but Golightly (1940) has shown that both are very abundant near the surface when *Metriocnemus* is absent, for it is in the top foot that most food is available. *S. perennis* larva has a tendency to go downwards. In spring time *L. lineatus* has a period of special activity and destroys almost entirely the surface *Phormidium* which is then loosened by the events of winter. This is washed down the bed in pulverized form and there is a tendency for worms and larvae to follow it. Two other forces tend to make the dominators of the upper zone go more deeply into the medium. One of these is winter cold, for at this season the worms are densest 8 in. from the surface (Reynoldson, 1939*a*) and *M. longitarsus* larvae also tend to go down in very cold weather. The other force is drying and, if this is gradual in onset, there is naturally a retirement of worms and larvae though pupae and eggs are caught and desiccated. If the onset is sudden, as occurs in hot bright weather when the sprinklers are stopped for a few hours, then the worms which are in the *Phormidium* or between it and the pebbles, and the eggs, larvae and pupae in the topmost few inches, according to heat and length of stoppage, are killed.

The records of fly trapping have been considered with reference to a meteorological record of monthly averages of rainfall, total rain and number of days with rain; hours of bright sunshine; number of relatively calm days with equable temperature as these favour mating; and the bed temperatures which reflect all meteorological components. The air humidity was not considered as being too complex combined with the working of the sprinklers. Fly index was examined against fly index also by months and for longer periods. Where it appeared on setting out the data that there might be correlation the factor was calculated. It may be said at once that the separate

weather components exert no apparent effect except summer sunshine, hardly separable from bed temperature, and summer rainfall. On the other hand, there is enough correlation with bed temperature and amongst the flies to justify the labour spent. These correlations show themselves always as discovering delayed effects on fly emergence, with the exception that current temperature and fly index are related in 4 months for *P. alternata* and in December for *P. severini*. The latter is probably accidental and, for *P. alternata*, the trapping has been known to depend partly on abundance and partly on activity. There is also negative correlation in June between *M. longitarsus* and current sunshine. The weather details required are shown in Table 3, and for the sake of the narrative the correlation coefficients are gathered into an appendix.

The influence of summer conditions and of winter conditions need to be taken separately and the former will be dealt with first.

Summer influence

In 1934, June was 0·5° C. above average, and July was particularly hot with 245 hr. sun. *M. longitarsus* was significantly below average in July and August, low in September, and significantly deficient in October and November from summer oviposition. *S. minima* was significantly in excess in August and September, with life cycles of some 35–40 days, and again in December from oviposition in early October. *M. hirticollis* was above average in September. *P. alternata* had been in significant excess through the hot summer with an activity-abundance response, but became significantly deficient in September and October though temperature was slightly above average, a fact which emphasizes the deficiency.

In 1935, June temperature was almost average while July was very hot, nearly 2° C. above average with 266 hr. sun and deficient rain, 0·73 in. This exceptional July does not exert the lasting effect on *M. longitarsus* developed from a hot June for reasons given later, and deficiency is found only in September, probably from July oviposition. The year appears to be one of balance for all the chironomids, as the significant deficiency of *S. minima* in December is accounted for by the fact that this was the coldest December in the record, 2·2° C. below average. This caused delay in pupation, for the fly numbers are well up in the next month, almost as cold. *P. alternata* had been in significant excess in August but sank to the reverse condition in the next 2 months, September being rather cool and October average. The deficiency is probably associated with the combined influence of the chironomids which, in the aggregate, were unusually abundant.

1936 is an instructive year. June and July were respectively the coldest of the period, the former with excess rain, 6·5 in., and the latter with average rain. These conditions are particularly suited to the surface habit of *M. longitarsus* (and to *L. lineatus*, but worm abundance was not assessed and only the fly is referred to in this section), and from average numbers through June to August it came to significant excess in September and October. Although the summer months were in some respects less suited than usual to mating and speed of development of the other chironomids, they were ideal for oviposition which takes place near the surface, and in all respects they were better than spring and autumn months in general during which both flies increase with marked success. *S. minima* had been average in May and July, slightly below average in June, but it went into a significant collapse in August from June–July oviposition and the collapse persisted till December. *M. hirticollis* had been in significant excess in May and June and maintained about average numbers till October. It then went into significant collapse and remained in a collapsed condition till the spring of 1940: recovery actually took place a little earlier but did not show in fly output till spring. Offspring from July to September oviposition give the exact point of the collapse and they were in contact with the excess of *M. longitarsus*. There can be no doubt that it was this excess of the rival species and not the cold summer that caused this remarkable decline in *S. minima* and *M. hirticollis*. *P. alternata* had been emerging in average numbers from May to August (June excepted) but showed significant excess in September and October and, though temperature would not account for this, some release from the pressure of chironomid larvae in the depths would do so. *P. severini*, however, does not show benefit from this release.

1937 calls for little comment. The summer was average in temperature, sun and rain. *M. hirticollis* remained uniformly low but the other four flies showed no deviation from the average.

In 1938, June was again cold and July 1° C. below average. There was deficient sun and 11 in. of rain in the 2 months. This is, therefore, again a suitable season for *M. longitarsus* which shows excess in September and October which would have significance if 1936 were excluded. *S. minima* was average in June but went into significant collapse in July from early stages that would have made contact with the excessive brood of *M. longitarsus*. It remained deficient till October but its numbers recovered in November which was 2° C. above average and so more suited to the activities of this fly than November usually is. *M. hirticollis* in the depths of its depression in May and June shows low numbers for the rest of the year. *P. alternata* shows deficiency in July and August, significant in the latter and probably connected with the excessive *M. longitarsus* larvae which matured in August. It recovered to significant excess in September to November, attributable to current temperature only in the last of these months. This is the same response noted in the similar year 1936 and is due to some release from pressure of the chrionomid larvae in the depths.

TABLE 1. *Monthly index of fly population, means and standard errors*

Low significance in italics, high significance in heavy type

	1934	1935	1936	1937	1938	1939	1940	1941	Mean
					Spaniotoma minima				
Jan.	**1361**	523	405	181	*165*	327	362	282	451 ± 136·4
Feb.	239	273	72	99	*57*	**517**	226	257	218 ± 52·8
Mar.	**327**	68	115	100	104	209	129	253	163 ± 32·0
Apr.	80	112	102	114	109	114	*33*	93	95 ± 9·8
May	442	**832**	530	448	413	**914**	403	442	553 ± 71·8
June	*511*	856	848	787	1237	**2055**	1261	**2322**	1235 ± 226·5
July	2456	(3533)	2768	2906	*896*	(4449)	2789	**4814**	2773 ± 509·1
Aug.	**7297**	6209	*2679*	6026	4565	**6843**	6678	4525	5605 ± 549·1
Sept.	**9650**	4335	*1802*	3918	2823	2328	**7550**	—	4630 ± 1107·5
Oct.	2863	2590	*1545*	3236	**2182**	2773	**7679**	—	4149 ± 993·2
Nov.	1483	1740	*683*	2223	2457	3059	**6356**	—	2571 ± 693·3
Dec.	**2760**	*268*	*434*	1154	1547	1918	**2935**	—	1574 ± 396·0
					Metriocnemus longitarsus, female flies				
Jan.	122	**218**	134	*45*	*16*	**204**	24	8	96 ± 30·1
Feb.	23	**135**	44	*6*	*4*	**81**	14	14	40 ± 16·3
Mar.	42	17	45	*17*	18	**86**	62	*20*	38 ± 9·0
Apr.	33	24	32	49	18	47	**68**	*8*	35 ± 6·8
May	89	103	93	64	20	93	93	**860**	179 ± 98·0
June	56	29	36	56	32	24	*12*	**101**	43 ± 9·8
July	*7*	(23)	25	22	12	(31)	*8*	**32**	18 ± 4·1
Aug.	*7*	18	17	26	9	**41**	7	20	18 ± 4·0
Sept.	11	4	**64**	22	30	18	*1*	—	21 ± 8·1
Oct.	*7*	21	**80**	24	38	15	*1*	—	27 ± 10·0
Nov.	*27*	79	71	80	**105**	**130**	*52*	—	78 ± 12·7
Dec	34	47	61	151	**308**	78	42	—	103 ± 37·3
					Metriocnemus hirticollis, female flies				
Jan.	26	**90**	48	1	4	2	1	7	22 ± 11·3
Feb.	1	**146**	0	1	1	0	1	7	20 ± 18·0
Mar.	2	**49**	4	1	1	1	2	5	8 ± 5·9
Apr.	1	**231**	6	1	**2**	1	1	1	31 ± 28·6
May	13	**61**	**86**	13	*2*	*3*	34	33	31 ± 10·5
June	40	58	**167**	11	*8*	*6*	46	**224**	70 ± 28·7
July	19	(50)	40	13	*1*	(4)	42	**116**	39 ± 16·8
Aug.	35	45	23	12	15	*3*	**169**	**104**	51 ± 22·8
Sept.	66	6	25	12	19	10	**117**	—	36 ± 15·4
Oct.	27	38	40	18	6	12	**86**	—	32 ± 10·1
Nov.	20	30	*3*	15	20	18	**75**	—	26 ± 8·7
Dec.	52	24	3	18	14	2	**194**	—	44 ± 25·8
					Psychoda severini, parthenogenetic				
Jan.	*1*	2	6	4	2	**14**	1	**20**	6 ± 2·5
Feb.	*1*	**93**	19	16	16	9	8	**81**	31 ± 12·4
Mar.	*57*	**590**	**709**	*12*	100	41	365	**524**	300 ± 99·0
Apr.	*46*	**1361**	484	*145*	233	451	**2182**	1149	756 ± 262·9
May	*167*	**1281**	**1549**	*560*	765	**1600**	558	867	918 ± 181·4
June	24	142	**1567**	110	560	135	43	223	351 ± 183·7
July	2	—	21	15	**214**	—	23	12	48 ± 33·7
Aug.	0	3	5	1	5	5	6	1	—
Sept.	0	1	1	1	1	1	1	—	—
Oct.	0	0	3	1	6	3	**29**	—	6 ± 3·8
Nov.	2	0	0	1	10	**30**	**20**	—	9 ± 4·4
Dec.	**16**	*1*	*2*	9	8	**12**	9	—	8 ± 2·0
					Psychoda alternata				
Jan.	0	3	0	1	11	**2**	0	0	—
Feb.	0	3	0	0	6	1	0	0	—
Mar.	4	3	0	0	62	1	1	0	—
Apr.	19	4	1	4	**219**	53	5	0	38 ± 26·5
May	130	*124*	264	**623**	157	**497**	376	*0*	271 ± 74·8
June	**986**	*78*	117	245	152	377	577	*51*	323 ± 113·4
July	**907**	(134)	229	152	52	(249)	208	56	267 ± 131·5
Aug.	**205**	**190**	118	120	*61*	121	150	154	140 ± 16·1
Sept.	*29*	*30*	**145**	79	**153**	19	49	—	72 ± 21·2
Oct.	*13*	*13*	**51**	27	**43**	33	33	—	30 ± 5·4
Nov.	9	2	0	9	**51**	10	1	—	12 ± 6·7
Dec.	**6**	0	**6**	2	1	1	1	—	2·6 ± 0·9

P. severini is interesting in this summer, since in the six Julys of which there is record only now does it appear in numbers and shows significant excess coinciding with the only occasion when *S. minima* is in a significant deficiency in July.

In 1939 temperatures in summer were about average, the season was deficient in sun, while rain was deficient in August and very deficient in September. It is now necessary to turn to the record of trapping of *M. longitarsus* larvae and pupae at the surface of the bed extending over 3 years. These being low at Leeds and this particular bed not employed. From 4 to 11 Aug. only four larvae were taken in the three traps, and for the rest of the month the average was only seventeen per trap-week. September with only 0·44 in. of rain gave five per trap-week and the barrage remained thin, the cold winter contributing its influence, till the following May. The emerging *M. longitarsus* were below average in September and October from the early August check but significantly high in August and November, showing that the surface location is not

TABLE 2. *Index of larval and pupal population of* Metriocnemus, *nearly all* M. longitarsus, *in surface zone. Weekly averages*

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1938	—	—	—	128	37	82	32	26	66	62	201	95
1939	71	143	246	78	334	175	—	10	5	9	23	6
1940	Frosted		8	20	255	70	10	1	13*	7	5	19
1941	5	Frosted	2	5	—	—	—	—	—	—	—	—

* These were 90% *M. hirticollis*.

TABLE 3. *Bed temperatures* (° C.), *summer rainfall* (*in.*) *and bright sunshine* (*hr.*) *at Knostrop, Leeds*

	1934	1935	1936	1937	1938	1939	1940	1941	Mean
					Temperature (° C.)				
Jan.	9·2	8·1	6·3	10·1	8·2	6·3	6·1	1·7	7·0
Feb.	8·4	7·8	5·6	9·5	7·6	9·3	4·1	4·6	7·1
Mar.	9·0	9·6	8·2	8·4	10·9	8·4	7·4	6·5	8·5
Apr.	10·4	9·9	9·4	11·7	10·6	10·7	9·3	7·1	9·9
May	12·8	12·1	12·2	14·1	12·0	12·2	13·5	10·6	12·4
June	15·7	15·0	14·4	15·4	14·6	15·0	16·8	14·5	15·2
July	18·2	18·3	14·1	16·6	15·4	16·6	16·3	16·6	16·5
Aug.	17·1	17·5	16·6	16·5	16·8	16·9	16·1	14·9	16·5
Sept.	16·4	14·0	16·7	15·7	15·9	15·9	14·0	—	15·5
Oct.	13·0	12·8	12·6	13·7	12·8	11·2	11·0	—	12·4
Nov.	11·0	8·1	8·0	10·8	11·8	11·0	8·4	—	9·9
Dec.	10·9	5·9	8·1	8·1	7·8	8·5	7·4	—	8·1
					Rainfall (in.)				
May	1·62	1·04	2·60	3·23	5·61	1·97	0·78	2·20	2·38
June	2·08	3·19	6·53	2·78	2·39	2·12	0·44	0·58	2·51
July	2·73	0·73	3·71	3·02	5·31	4·66	4·40	—	3·51
Aug.	1·92	2·15	2·44	1·24	5·70	1·73	0·43	—	2·23
Sept.	3·51	5·87	2·75	1·47	2·56	0·44	1·86	—	2·64
					Sunshine (hr.)				
May	183	255	170	178	163	177	206	100	179
June	181	186	184	174	187	207	273	165	195
July	245	266	163	164	145	127	134	—	178
Aug.	174	226	184	164	156	129	160	—	170
Sept.	148	152	92	132	94	84	129	—	119

figures do not always bear a relation to the index of emerging flies partly because of mortality, partly because the larvae are deeper when food is scarce, while they tend to descend somewhat during heat and in very cold winters. According to the degree of descent the larvae make, or according to abundance and mortality, the barrage they form is thinned. They showed normal abundance from April 1938 to June 1939, there being no record in July 1939. In the first week of August they averaged twenty-eight per trap. The sprinklers then stopped for some days, sewage flow over the August holidays essential to the success of this species. *S. minima* was in significant excess from May to August, distinctly deficient in September and October, but recovered in the next 2 months. It does not therefore reciprocate to the emerging numbers of *M. longitarsus* as in 1936 and 1938. The point is developed in detail because it is certain that it was in this autumn, when the *M. longitarsus* barrage was dispersed, that *M. hirticollis* started its recovery. This does not show in the autumn record when its numbers are about the same as in the two previous years, but it does show in May and June 1940 as the

flies then emerging must have been the product of autumn breeding, the winter months ensuing being much too cold for imaginal success. Thus it is not so much the number of larvae of *M. longitarsus* present in the bed that influences the abundance of the other chironomids but rather the density of the barrage that it forms near the bed surface. For *P. alternata* this was an average year, but *P. severini* shows significantly high numbers in November and December, again showing probably the importance of the barrage with confirmation coming in the next year.

1940 was characterized by the abnormally high temperatures of May and June with most excessive sun and deficient rain, 1·22 in. in all. *M. longitarsus* as a fly was in significant excess in April owing to the cold winter giving accumulated emergences as in 1941, and it was average in May with a fully recovered larval barrage, 255 per trap-week. From June to November it went into a significant collapse as a fly and the larval barrage was almost absent since, of the thirteen per trap-week in August, 90% were *M. hirticollis* which has usually been scarce in these traps. *S. minima* was normal through June and July, in excess in August, and in significant excess up to the end of the year and, if the retarding effect of the very cold winter could be taken into account, it is really in excess till the following March. Instead of reaching its zenith in August, as is usual at Knostrop, it behaved more as it does at Barnsley where there is no *Metriocnemus* to interfere with its increment (Lloyd, 1937) and continued to build up its population to the onset of winter. This behaviour was in spite of the temperatures being rather below average from July to the end of the year and so rather against its specially rapid increase. *M. hirticollis* which showed some recovery in May emergences appeared in significant excess in August, and continued to do so through two complete generations till December though, again, temperature conditions were not specially favourable, and the only novel factor was the breaking of the *M. longitarsus* barrage. *P. severini* with significant excess in October and November also responded to the thin barrage, but *P. alternata* showed average numbers through the year.

In 1941 the fly record was continued till August and the larva trapping till April. The winter was the coldest of the record and its retarding effect is very evident in accumulated emergences which, in part, are responsible for the significantly excessive numbers of the three chironomids in late spring and early summer. This confuses the study and the trapping was not continued late enough for the effects of summer influence to emerge distinctly.

Winter influence

In the catches of the first 6 months of the year it is considered that there is no evidence of interspecific competition amongst the flies before May or June. The factors which determine their abundance in these months will be given.

With *S. minima* the dominant influence appears to be the abundance of breeding stock available towards the end of the preceding year. The numbers trapped from October to December in 7 years are set out below in order of magnitude with the corresponding figures for the following 6 months:

Year ...	1936–7	1935–6	1938–9	1937–8
Oct.–Dec.	2662	4598	6186	6613
Jan.–June	1729	2062	4136	2085

Year ...	1934–5	1939–40	1940–1
Oct.–Dec.	7716	7750	16970
Jan.–June	2664	2414	3649

The third pair forms the only striking deviation, and this is due to the favourable November of 1938, 2° C. above average, which led to significant excess in February which, in turn, was 2° C. above average and led to significant excess again in May and June. Apart from this there is evidently much reduction of population during the winter months but mortality does not seem to vary greatly in different seasons.

M. hirticollis shows the same general rule but not with the same approximation to a common ratio. The trappings are set out as before graded for autumn numbers above:

Year ...	1939–40	1938–9	1936–7	1937–8
Oct.–Dec.	32	40	46	51
Jan.–June	85	13	28	18

Year ...	1935–6	1934–5	1940–1
Oct.–Dec.	92	99	355
Jan.-June	311	635	277 (+)

Owing to the retarding effect of the 1941 winter probably the last figure should be increased by about 200, the number trapped in July and August, as these must have been from autumn breeding because of the thermal requirements of the species (see Table 5). Therefore it seems again that the amount of breeding stock in autumn, rather than events in winter, is the chief factor in determining winter-spring abundance. This explains why the fly cannot recover from a summer collapse with the ease shown by *M. longitarsus*, and why it must wait for a summer when the barrage of the latter is thinned before it can recover. Many favourable periods of weather occurred between 1936 and 1939, but *M. longitarsus* did not fail in this interval.

The events of winter are dominant in regulating the numbers of *M. longitarsus* and *Psychoda* spp. in spring, for there is no apparent relationship between their autumn and spring abundance. For instance, with *P. severini* a population index of one in autumn 1935 was followed by an aggregate of 4345 from January to June, while 58 in 1940 was followed by 2864 in 1941. *M. longitarsus* indices in autumn and spring are less discordant but rather resemble the counts given by Roebuck (1936) for the larvae of the carrot fly where the second generation of the year may be much larger, or much smaller, than the first.

P. alternata has high developmental thresholds, a capacity for mating in complete darkness, and a pronounced temperature-activity response influencing trapped numbers; one would, therefore, expect that warm winters would be favourable to spring abundance in the traps and this is generally so. For instance, the warm winter of 1937–8 was followed by unusual early abundance,* while the cold early months of 1936, 1940 and 1941 were all followed by retardation and in the last very cold season by reduction in abundance as well, which may have been due in part to the harmful effect of cold and in part to an unusual abundance of all the competing chironomids.

M. longitarsus and *P. severini* have low developmental thresholds and progress is steady at 1–2° C. The fully favourable zone for the former is 5–18° and for the latter 8–18°, i.e. the zone without detectable temperature mortality (Lloyd *et al.* 1940). Therefore, one would assume that warm winters would lead to their greater abundance, and there is no doubt that if either were alone in the bed this must be the case. Yet the reverse is found as will be seen in Table 4. Here the temperatures of January to March are set out in relation to their averages over 8 years. The trapped flies are set out as percentage excess or deficiency of the aggregate catches from January to June and the standard errors are given, significance being italicized.

Table 4 shows that there are three warm winters each with at least one month 2° C. above average, two which can be classed as average (though 1939 has February 2·2° C. high), two cold with all months below average, and one year very cold. In the three warm seasons and 1939 *P. severini* is below average, twice significant, in three cold winters and in 1935 it is above average, once significant. *M. longitarsus* is below average in each warm season, once significant, and significantly high in the very cold 1941. *P. alternata* tends to the reverse condition. *M. hirticollis* and *S. minima* tend to be below average in the warm seasons, but this is accidental, breeding stock in the relative autumns being low. Further evidence on this point is given in the Appendix.

The anomaly is that when climatic conditions bring the two species of winter-breeding flies under temperature conditions which, in the laboratory, are fully favourable they do less well than at temperatures which, experiment indicates, slow down their life cycles and cause mortality in the case of *P. severini*. It is believed that this is explained by reference to the behaviour of the enchytraeid *L. lineatus*. According to Reynoldson (1939 *a*, *b*) this worm breeds in the beds chiefly in the winter months. It feeds especially on the *Phormidium* surface growth which it almost completely destroys some time in spring, causing deprivation to the larvae, many of which then pass to the floor of the bed and are washed away (Lloyd *et al.* 1940). Weather conditions, on the other hand, cause the growth to peel off in large fragments which the medium retains, and these are available for larval food near the surface. Reynoldson (1943) finds that the developmental thresholds for *L. lineatus* are at about 3° C. and the thermal requirements are such that it makes very slow progress below 7°. At 4·5°, incubation and growth occupy 200 days, at 7·5°, 170 days and, at 10°, 110 days are required. These latter temperatures are about the limit of cold and warm in the beds in winter, and it is evident that in the seasons classed above as warm the worm could complete one generation and start a second cycle. In the cold winters, however, a cycle could not be completed till spring was well advanced, while 1941 must have been very retarding to the worm. Its descent in cold weather to a depth of 8 in. is a contributory factor, for then it cannot get at the surface growth but is more dependent, like the larvae, on fragments of *Phormidium* detached by frost. This means that after warm winters there is a tendency for the disintegration of the *Phormidium* to occur earlier in the year and the period of deprivation to reduce the numbers of larvae and pupae in the beds at an earlier date. The greater warmth will no doubt increase activity of the larvae

TABLE 4. *The relationship between winter temperature and fly emergence from Jan. to June. Years arranged to separate mild and severe winters*

	Temp. ° C.			Fly indices				
	Jan.	Feb.	Mar.	*M. longitarsus*	*M. hirticollis*	*S. minima*	*P. severini*	*P. alternata*
Av. ...	7·0	7·1	8·6	100	100	100	100	100
				%	%	%	%	%
S.E.				±23·0	±42·3	±10·8	±20·5	±21·6
1934	+2·2	+1·3	+0·4	−12·5	−54·2	+ 9·1	*−87·5*	*+76·7*
1937	+3·1	+2·4	−0·2	−43·2	−84·5	*−36·3*	*−64·1*	+35·4
1938	+1·2	+0·5	+2·3	*−74·1*	−90·1	−23·2	−29·0	− 5·8
1939	−0·7	+2·2	−0·2	+ 4·2	−92·8	*+52·4*	− 4·7	+44·4
1935	+1·1	+0·7	+1·0	+25·9	*+250·3*	− 1·8	+46·9	−66·6
1936	−0·7	−1·5	−0·4	− 8·0	+71·6	−23·4	*+83·5*	−40·7
1940	−0·9	−3·0	−1·2	−34·6	−53·1	−11·0	+33·3	+48·8
1941	−5·3	−2·5	−2·1	*+142·2*	+52·8	*+34·5*	+21·3	*−92·1*

* Even more pronounced after the mild winter of 1942–3.

also, and this will be a contributory factor but it is *L. lineatus* that initiates the sloughing. It is interesting to find the worm drawn into the picture in this specific manner, but unfortunate that the notes on the surface growth have not been sufficiently regular to allow of the placing of the dates of its breaking up in the various years.*

S. perennis is usually a scarce fly at Knostrop, and its annual incidence per foot year through this record has been 17, 27, 300, 32, 6, 7, 6, the high number being in 1936. Most of its emergences occur about May and it does not appear through the summer when it is in prepupal diapause followed by a slight autumn flight. The habit of descending to pupate and the diapause, which none of the successful Nematocera show in the beds, are thought to account for the failure (Lloyd *et al.* 1940). Nothing can be said definitely about its fluctuation, but attention can be drawn to the character of the winter of 1935–6 which preceded its period of abundance. Cold weather started in November, and December was the coldest of the record, while January to March were all below normal. This was, therefore, the longest cold spell of the record though not with the most intense cold. As winter obviously suits the larva best it seems likely that its year of abundance was associated with a long wintry season.

Fluctuations of *Psychoda* species

There is a tendency for the annual abundance of the two species to show negative correlation. The years' totals are arranged below graded for *P. severini*.

Year ...	1934	1937	1938	1939
P. severini	316	875	1920	2301
P. alternata	2308	1262	968	1364
Year ...	1940	1935	1936	
P. severini	3245	3474	4366	
P. alternata	1401	584	931	

Though the two species must be in competition, the apparent relationship is thought to be due to the fact that cold winters suit the former for reasons of competition while warm winters suit the latter for physiological reasons which outweigh other disadvantages. Evidence has already been given that their abundance and the curtailment of their seasons of prevalence, especially the sharp decline of *P. severini* in early summer, are the result of the combined activities of the chironomids (Lloyd, 1937; Lloyd *et al.* 1940; Golightly, 1940). It has not hitherto been possible to separate the influence of *M. longitarsus* from that of the more deeply seated larvae with any precision but this can now be done.

In 1934, *P. alternata* had significant excess in June to August and significant deficiency in the next 2 months. Temperature was very favourable in summer but September and October were also favourable and current temperature plays no part in the collapse. Extraneous events were that *S. minima* was significantly deficient in June while *M. longitarsus* was deficient from August onwards (effect of summer heat), but in August and September *S. minima* developed significant excess (effect of *M. longitarsus* collapse). Excessive *S. minima* can thus account for the collapse of *P. alternata* but the ultimate cause is June temperature. 1935 was the worst year for *P. alternata*, apart from the incomplete 1941. The early part of the year should have been favourable, but all the other four flies showed some excess, especially *M. hirticollis* and *P. severini*. There is little doubt that *P. alternata* was largely squeezed out by combined pressure. It shows significant deficiency in May and June (both favourable), incidence in July not known, significant excess in August (the hottest of the period), but significant deficiency again in September (very cold), and October an average month. There is no indication of special pressure of the chironomids after May but the unfavourable September would check it. In 1936 it showed average numbers through the summer but significant excess in September (the hottest of the period) and October (average), but there is no need to attribute this excess of *P. alternata* to current temperature because the same late excess occurred in 1938 with average temperatures. In each case it was associated with significant deficiency of *S. minima*, excess of *M. longitarsus* and, ultimately, with cold Junes. In 1937 there is nothing remarkable about *P. alternata* except a significant excess in May associated with high current temperature and a warm preceding winter with significant deficiency of *M. longitarsus* and, still more, of *P. severini* which was scarcer than in any other year except 1934, when it was again followed by an excess of *P. alternata*. In 1938 the winter was not unfavourable to *P. alternata* and March was the most favourable of the period. It showed excess in March and April for which current temperature would account. The later part of the year has been dealt with. In 1939 it showed nothing remarkable except a significant excess in May for which current temperature will not account, nor will deficiency of *M. longitarsus*, *S. minima* and *P. severini*, since each was in excess either before or during May. The only event of the year common with 1937, when *P. alternata* was also in excess in May, was that February was 2° C. above the average and the developmental rate would have allowed these May flies to have come from February oviposition, about 80 days cycle at 10° C. It may be noticed, however, that these three seasons when *P. alternata* shows significant excess in an early month cover the years when one competitor, *M. hirticollis*, was much depressed. In 1940 the high trappings of *P. alternata* in May and June can be associated with high current temperature. In 1941

* Strongly confirmed in the mild winter of 1942–3. A bed stopped for 3 months in autumn lost all *L. lineatus* and in March has excess *Metriocnemus* spp. with no *P. alternata*. Beds continuously working have excess *L. lineatus* and *P. alternata* with deficient *Metriocnemus*.

the exceptional winter cold would induce undue mortality, which would account for spring deficiency, and the effect of the excess of chironomids cannot be distinctly separated from this.

As *P. severini* is in any case scarce at Knostrop after the middle of the year the causes of its annual fluctuations seem to be mainly bound up with the events of winter and spring surveyed above. Attention may be drawn to the fact that it extends its season of abundance by a month or two in a significant manner in 1936 and 1938, the 2 years with cold Junes when *P. alternata* also extended its abundance into autumn. These are the 2 years when *S. minima* showed significant deficiency and *M. longitarsus* significant excess. At the close of the year, however, *P. severini* shows, though with low numbers, significant excess in December 1934, in November and December 1939, and in October and November 1940. The first of these occurred in the hottest December of the record, but the excess is not attributed to this because the fly is very active at much lower temperatures, e.g. January to March 1941. 1934 and 1940 were years of late significant excess of *S. minima* and in 1939 it showed average abundance. But 1934 and 1940 were the 2 years when *M. longitarsus* showed significant deficiency, and in 1939 it has been shown that though the larvae were abundant they were not forming the usual barrage in autumn (when these late *P. severini* had their origin) because of machine stoppage in August and a dry September. Thus an anomaly develops amongst the correlation coefficients shown in the Appendix, for *S. minima* and *P. severini* show significant negative correlation for the May or June to December catches but from October onwards the correlation becomes a significant positive. The probable explanation is that through the summer months, the barrage of *M. longitarsus* larvae tending to be less dense generally, *S. minima* whose larvae are far more numerous, assumes greater importance as the antagonist of *P. severini*; but with the coming of the cooler months and the normal closing of the barrage *M. longitarsus* assumes greater importance in control. The summer and autumn correlation between *S. minima* and *M. hirticollis* is still more striking. Both are in antagonism to the barrage-forming larvae so, though they must be themselves in competition, they show very strong positive correlation in their summer and autumn abundance.

Discussion

In previous studies of the fauna of the bacteria bed (summarized by Lloyd *et al.* 1940) it has been shown on a variety of evidence that the effects of interspecific competition amongst the dominant insects can be demonstrated by (1) restricted seasonal incidence, (2) positional trapping on a long bed with travelling sprinklers, and (3) relative abundance at different localities where the composition of the fauna differs. It has also been shown that *L. lineatus* intervenes as a deprivative competitor and as initiator of the spring sloughing which causes much loss to the early stages of the insects. The presence of chironomids reduced the numbers of psychodids and the length of their period of abundance was curtailed. Some evidence of antagonism amongst the chironomids was also produced but was less decisive. There was, for instance, some evidence in the positional trapping that the abundance of *M. hirticollis* was inverse to that of *M. longitarsus*. In the present study an attempt is made to discover whether this interspecific competition influences the annual fluctuations of the insects, or what other factors are responsible for these, and in this attempt some new information about the mechanism of the competition has come to light.

The whole question of annual fluctuation has recently been reviewed by Bodenheimer (1938) who points out that fluctuations amongst insects and some other animals are commonly of the order of 1 : 100 or 1 : 1000. After a critical consideration he concludes that though variations in numbers of predators and parasites or disease may play a part, the main determining factor is the weather. The fluctuations observed in this short period of seven completed years have not been of such dimensions and are as follows: *M. longitarsus* 1 : 2·2 (perhaps 1 : 4 if 1941 had been completed), *M. hirticollis* 1 : 13, *S. minima* 1 : 3, *P. alternata* 1 : 4, *P. severini* 1 : 14, while *S. perennis*, which after those named is the chironomid most at home in the beds, varied only as 1 : 50. No doubt the stability of the environment as opposed to extreme variation in conditions found usually where fluctuations have been recorded, is responsible for this restricted range. This stability embraces not only physical factors and food supply, approximately the same amount of basic food reaching the bed each month each year, but also the animal enemies of the insects studied. Spiders form the most important group and they have varied annually as 1 : 4 as judged by trapping where they are as 1 : 700 emerging flies through the whole period. A few parasitic Hymenoptera breed in the insects on the beds but the emerging forms are only as 1 : 12,000 emerging flies and their annual fluctuation has been as 1 : 11.

The fly numbers have been arranged as weekly averages for the successive months and studied with reference to a meteorological record for current and delayed effects. Similarly, the species have been set against one another by months and for longer periods. No weather components, except sunshine and rainfall in a few instances, have been found to exert visible effect but there is marked correlation with bed temperature. This is not due to a simple temperature-activity response except in the case of *P. alternata* where it is partly so, for in 4 months of the year there is positive correlation between this fly and current temperature. Otherwise the effect is delayed and often indirect. The implications of the

findings have been partly discussed in the previous narrative but some points need fuller treatment.

The larvae of *M. longitarsus* tend to form a barrage in the top foot of the bed, and this is denser or thinner in autumn according to summer sunshine and rainfall combined with stoppages of the sprinklers. When June is hot and dry the population is reduced in autumn and the barrage is thin. When June is cold and wet, conditions are favourable for the late summer and autumn brood and the barrage is denser. The barrage may also disperse through dry conditions in autumn without evident reduction in the brood. Such might also occur at other times, but it is not evident in the record. When the barrage is denser than usual in summer *S. minima* shows a following period of depression but recovers quickly, at any rate by the following summer. When the barrage is thinned out in summer a period of elevation for this fly follows. *M. hirticollis* is in similar case, but the onset of depression, or of elevation, is not so close to the altered barrage condition as in *S. minima*; it does not recover from collapse with the facility shown by the latter species.

The summer and autumn abundance of the psychodids is also related to barrage density but with an opposite tendency. When the barrage is dense after a cold wet June and *S. minima* larvae (and perhaps those of *M. hirticollis*) are thereby reduced in the depths, *P. severini* is not curtailed so abruptly in summer and *P. alternata* extends its abundance into autumn to such an extent that a strong positive correlation develops between its index of abundance and that of *M. longitarsus* (see Appendix). Hot dry Junes might be expected to be favourable to *P. alternata* but are followed by numbers lower than usual because of the elevated state of *S. minima*. Towards the end of such years, however, *P. severini* from autumn breeding becomes significantly more abundant than usual, though still with low numbers, in spite of abundance of *S. minima* larvae. This confirms the belief that *M. longitarsus* is the main controlling force of *P. severini* in autumn though *S. minima* mainly causes its abrupt curtailment in summer. The developmental threshold of the larva of the latter is 5·5° C. and, as the beds cool towards winter, its activities will be reduced though not stopped entirely in the beds except under most extreme conditions. *M. longitarsus* larva, however, has a threshold of 1·7° C. and is very active through the winter. Therefore, it seems that *P. severini* can increase against an extra abundance of *S. minima* larvae in late autumn but not against a barrage of *M. longitarsus* larvae of normal density.

The effect of the barrage density on *S. minima* is not evident for 1–2 months and on *M. hirticollis* does not show till 2–3 months have elapsed and, in one instance with the latter, the whole winter intervenes between effect, increased emergence, and cause, dispersed barrage. Study of the lengths of the life cycles against current temperatures (Table 5) show that it is the establishment of a brood that is interfered with rather than the progress of established brood. A severe attack on pupae for instance should reveal itself in a current month since pupation is so short. It is natural that the chironomids should oviposit in the top of the bed for the act follows flight. This is also the case with *P. alternata* in the warmer months, and *P. severini* also leaves the bed on eclosion before returning to oviposit unless deterred by very cold weather. Leaving the bed, however, is not imperative on either *Psychoda* so some of their eggs may always be deposited deeply. It is believed that this natural tendency for most eggs, in summer and autumn at least, to be placed near the top of the bed is a crucial point in the competition. This is the zone preferred by *M. longitarsus* larvae and they devour the eggs of the other species very eagerly in laboratory tests (Lloyd *et al.* 1940). They will also devour pupae and the larvae of psychodids and, no doubt, do this also in the beds, but the egg destruction seems to reveal itself whilst pupa destruction does not do so.

The question arises why is June the critical month in competition? The reason is found in the timing of the life cycles shown in Table 5. These are all from observed data except for *M. hirticollis* at 6–7° C. which is calculated (Lloyd, 1937; Lloyd *et al.* 1940). There is a mating bar for *S. minima* at 10° C. and probably one for *M. hirticollis* at about the same level. *P. alternata* will mate at about 7° C., flight not being necessary though a flutter may precede mating. The photofugic habit as daylight strengthens causes clustering sufficient to bring the sexes together. The mating temperature limit of *M. longitarsus* is not known but it must be low as reproduction takes place in cold winters. This species can complete its cycle after oviposition in an ice chest. *P. severini* can carry through the whole cycle in an ice chest but mortality is great and the flies produced tend to be crippled.

TABLE 5. *Timed complete life cycles of the flies in days at graded temperatures*

Temp. ° C.	*M. longitarsus*	*M. hirticollis*	*S. minima*	*P. severini*	*P. alternata*
2– 3	243	—	260	200	—
6– 7	153	(200)	103	90	140
10	94	123	80	60	80
15–16	49	49	43	36	35
20–21	26	42	29	26	22

The flies though emerging continuously through their seasons are subject to peak periods which will be distinguished as flights. The result of these timings is that *M. longitarsus* from early autumn oviposition produces a midwinter flight from which it establishes successfully a large brood which leads to a second flight in May. The offspring from this is well established in June. *M. hirticollis*, however, from an autumn flight cannot produce a flight in

winter unless conditions are quite exceptional, such as occurred in 1934–5 when December had a temperature of 10·9° C. Thus, its larvae are overtaken by the cold months and as its larval threshold is about 3° C. and its thermal requirements about 660° C. (*M. longitarsus* larva with about 1·7 and 440° C. respectively) it makes very slow progress in winter. The result is that its flight from autumn eggs does not ordinarily take place till June and this flight places its eggs amongst the growing brood of its rival. In the next flight in autumn the same precedence will occur (the insects now taking about the same time) and, if *M. longitarsus* has had a successful season, competition will be intensified as in 1936 and 1938. It is in the next flight in late autumn that the effects of the reduction become evident. *M. longitarsus* with a barrage thin through lack of numbers, or dispersed, fails to exert noticeable control and increase of *M. hirticollis* becomes possible. Several cases where success in competition depends on the timing of life cycles are known amongst parasitoids (Imms, 1931).

S. minima with its quicker life cycles is in rather better case and it is always emerging in reasonable numbers through the winter. Even in the bitter January of 1941 it continued to emerge into traps buried in snow and the index for the month was 282 flies. Therefore, though most of the winter flies are wasted, some are always present to take advantage of a warmer spell. A rather sudden fall of temperature that ordinarily occurs in autumn tends to split the flights, with the result that two or even three successions of generations or flights of this fly are running. A rather sudden rise of temperature in spring tends to telescope the offspring from a whole series of separated ovipositions (Lloyd, 1941). The result is that there is a large flight in May, and a foundation for the year's abundance is laid down ahead of the period of great activity of the larvae of *M. longitarsus* produced by its May flight. It is, therefore, in subsequent flights that the influence of the barrage is exerted with an effect sufficient to show in emergence numbers. It is evident that *S. minima* should recover quickly from a decline against normal numbers of *M. longitarsus* since its lower thermal requirements allow it to catch up again in winter with a facility impossible to *M. hirticollis*. In exceptional years the initiation of these events might be earlier or later than June but, in 7 out of the 8 years covered, the spring flight of *M. longitarsus* was at its maximum in May (see Table 1) so June has been used categorically. By this theory the course of events in the later half of the year is explained to a satisfying degree.

In *S. minima* and *M. hirticollis*, abundance in the first 6 months is generally proportional to that of the previous autumn and the fluctuations of the whole year are traceable to June conditions. This is not so for the other flies. *M. longitarsus* has numbers erratically divided by June or by December. *P. severini* has nearly all its emergences in the first 6 months without relationship to autumn abundance. This is also true of the scarcer *S. perennis*, and its only year of abundance was preceded by the longest winter in the record. Lack of relationship between autumn abundance and that of the following spring is also found in *P. alternata* and the reasons for the size of its spring flights seem clear. Winter and spring temperatures are the main controlling factor as regulating mortality in winter, speed of development and spring activity. If there were any satisfactory method of showing the combined effect of the other insects it could probably be shown that the density of these is also a regulating factor. In 1935, for instance, the others abounded and though the winter had been good for its survival *P. alternata* failed significantly in spring. Again, in 1941, its failure until July seemed greater than the cold demanded and the chironomids were all in excess. If the record from other works is examined there is no doubt about the reducing influence of the chironomids. Golightly (1940) records *P. alternata* emerging in numbers through the winter at Huddersfield where the beds are only slightly warmer than at Knostrop. The difference is so great that it can only be attributed to presence or absence of chironomids.

The fluctuations of *P. severini* and *M. longitarsus* in the earlier part of the year are shown above to be related to winter temperature and the mechanism of the relationship is sufficiently discussed (see p. 53). Briefly, *L. lineatus* breeds more rapidly in warmer winters and is nearer to the surface *Phormidium* which it destroys. This leads to an earlier destruction of the surface growth and so, it is suggested, to an earlier period of spring deprivation for the larvae. Consequently a reduction in numbers of the winter breeding flies after warmer winters is evident. There is no evidence of interspecific competition amongst the flies developing before May with the exception of *P. alternata* just mentioned. It may be supposed that *P. severini* has an advantage over *P. alternata* in this because of its much quicker winter cycle (see Table 5). Life in general in the bed is more leisurely in winter than during the rest of the year while the medium is not being constantly reloaded with fresh ovipositions to the same extent. Evidence has been given that egg destruction is one of the main developments in the competition.

A remarkable feature of insect fluctuations is found in gradations. An insect builds up its population through a series of years and then more or less suddenly collapses to minimal proportions. The collapse is generally attributed to the arrival of weather conditions unsuitable to the species concerned rather than to population pressure or other biotic factors (Uvarov, 1931; Bodenheimer, 1938). In this short series of seven completed years, gradation is very marked for *M. hirticollis* and distinct for *S. minima*. It does not appear for *M. longitarsus* in the yearly totals but does so from July to December. The

gradations are shown in the following figures, the timing of the annual cycles being such as to take maximum advantage of the delayed effect in competition:

Year ...	1934–5	1935–6	1936–7	1937–8
M. longitarsus				
July–Dec.	93	192	318	325
M. hirticollis				
Nov.–Oct.	846	493	89	92
S. minima				
July–June	29,173	20,737	11,650	21,548

Year ...	1938–9	1939–40	1940–1
M. longitarsus			
July–Dec.	502	313	111
M. hirticollis			
Nov.–Oct.	76	519	786
S. minima			(+2 months)
July–June	18,606	23,784	37,636

The ultimate cause of the gradation in this case is the weather of June but this does not act directly on all three species. It affects profoundly the prosperity of *M. longitarsus*, the dominant species, because it is able to occupy the best position in the bed when weather favours it. The gradation in the other two depends on the prosperity of this dominant species. Thus the gradations in these are intimately connected with interspecific competition.

The reason why the effects of competition and its mechanism can be explained with some exactitude for the sewage bed fauna is that the populations of the components can be assessed with precision. A good deal of knowledge about each important member has also been gained by experiment and observation. To what extent similar effects might be expected in other types of fauna is worth consideration. It seems unlikely that there can be anything nearly parallel in fully exposed communities of animals. Something of the kind would appear to take place in the carcass (Holdaway, 1930) and the timing of life cycles is known to be of importance amongst parasitoids in competition in a single host (Imms, 1931). It would seem that competition much like that now explained must occur in the teeming life of shallow fresh water but its study would prove infinitely more complex because of the variety of forms involved and difficulties in assessing populations over continuous periods. Many of these forms would not lend themselves readily to laboratory study and this impediment has not been encountered with the animals of the bacteria bed yet studied.

Though under single authorship this paper is the result of teamwork and thanks are due to Prof. E. A. Spaul for encouraging students to take part in it. Each of these has contributed some salient point to the investigation without which the general course of events could not have been traced. Several have assisted in maintaining the almost continuous record. Mr G. Smeal has discussed points in statistical treatment. The meteorological record was provided by Mr H. W. Ricketts from the Leeds City Records but as the instruments were destroyed in March 1941 this was completed by Dr J. Grainger from the Huddersfield record. Officers of the Leeds Sewerage Department have afforded facilities and especially Mr J. T. Thompson has continuously assisted the work.

APPENDIX

The problem was first explored by searching for correlation between fly output and current physical and biotic factors. This proved abortive as no such correlation exists apart from a few unimportant instances. The totals for the successive years were then set against conditions of December and January respectively; then the totals from February to December against conditions of February and so on through the year eliminating one month at a time. In this way it was seen that while current events had only a secondary influence on fly output the influence of bed temperature in a certain month might exert a far reaching effect. It was seen that the temperature of June affected the behaviour of four of the flies up to the end of the year; that in two species this influence continued through to the following spring; and that in the other two species winter events negatived the influence of June conditions. The year was therefore divided at June and taken in the same manner through two 6-month periods. Interspecific relationships were examined for the half years and also for the whole years reduced in each case by successive months. A great deal of significant correlation was discovered in this way and a general idea of the course of events developed. An attempt was made to write up the theory on this basis but certain difficulties arose. For instance there appears to be negative correlation between *M. longitarsus* and *S. minima* extending from the February to December aggregates to the September aggregates. There is, however, positive correlation between these insects in the February to May totals for 1934–40 ($n=5$, $r=+0{\cdot}77$) and it was difficult to explain at what point in the year true negative correlation started. The brevity of the period dealt with and the great range of variation in abundance in different years also caused the coefficients in some cases to be unduly weighted by a single exceptional year especially in the case of *M. longitarsus*. Again with these two flies the output from July to December set against the June temperatures give for $n=5$ for *M. longitarsus* $r=-0{\cdot}72$ and for *S. minima* $r=+0{\cdot}96$,

while the interspecific relationship is expressed by $r = -0{\cdot}73$. The effect of June temperature thus appears to be stronger on *S. minima* but on biological grounds the effect on *M. longitarsus* can be explained while that on *S. minima* is not understandable except by interspecific competition. The figures cover three to four generations and there is no distinct relationship numerically between the first of these which might be influenced directly by the weather of June and the later ones which obviously could not. June weather, however, can deplete the stock of *M. longitarsus* which gives rise to the early autumn flight about the time when *S. minima* should be reaching its zenith in the year. The author believes that the apparent disparity in the coefficients is due to the fact that the fly index of *S. minima* more nearly represents the larval density than in the case of *M. longitarsus* which with its surface tendency suffers a great and erratic mortality in the pupa state through the summer due to bright periods during stoppages of the sprinklers. Thus the strong correlation coefficient for June temperature and subsequent *S. minima* output is believed to express no more than the interspecific effect, and to do so more exactly than the correlation coefficient between the indices of the species.

After some discussion with Mr G. Smeal on some of these difficulties a different treatment was adopted and the paper was given its narrative form through the years, reliance being placed on monthly averages and significant differences. The whole course of events then became clearer but the correlation coefficients still remain of use and are several times referred to in the paper. For this reason they are collected in the following tables, all above 0·50 being given. Levels of significance are as follows (Fisher, 1928):

	n	P ·1	P ·05	P ·02	P ·01
7 years	5	·67	·75	·83	·87
8 years	6	·62	·71	·79	·83

Correlation with current conditions

Bed temperature shows correlation with fly index in 4 months with *P. alternata* March +·75, May +·66, June +·69, for these $n=6$, and in November $r = +{\cdot}71$, $n=5$: also with *P. severini* in December $r = +{\cdot}82$, $n=5$, but this is believed to be accident.

Current sunshine shows negative correlation with *M. longitarsus* in June, $r = -{\cdot}69$ for $n=6$. There is no correlation between the fly indices and current rainfall or wind conditions.

Correlation with conditions of a previous month

No correlation is found with any condition except bed temperature. The year is divided into two periods and correlation is calculated between a monthly temperature and the aggregate of fly indices for that month and the succeeding part of the period.

Temp. of ...	*M. longitarsus*	*M. hirticollis*	*S. minima*	*P. severini*	*P. alternata*
		Jan. to June, $n=6$			
Dec.	—	—	—	—	—
Jan.	−·80	—	—	−·55	+·58
Feb.	—	—	—	−·65	—
Mar.	−·73	—	—	—	—
Apr.	−·83	−·62	—	—	+·65
May	−·69	−·57	−·61	—	+·76
June	—	—	—	−·56	+·72
		July to Dec., $n=5$			
June (July to Dec. fly)	−·72	+·86	+·96	+·59	—
July	−·60	—	+·52	—	—
Aug.	—	−·55	—	−·57	—
Sept.	—	−·58	—	—	—
Oct.	—	−·57	—	−·85	—
Nov.	+·61	—	—	—	+·72
Dec.	—	—	—	+·82	+·66

Correlation between the species

The calculations are based on annual aggregates reduced by successive months. For these $n=5$.

	M. longitarsus against			
	M. hirticollis	*S. minima*	*P. severini*	*P. alternata*
Jan.	—	—	—	—
Feb.	—	−·68	—	—
Mar.	—	−·64	—	—
Apr.	—	−·73	—	—
May	—	−·79	—	−·59
June	—	−·74	—	−·59
July	−·66	−·73	—	−·55
Aug.	−·61	−·66	—	—
Sept.	−·58	−·61	—	+·85
Oct.	−·53	—	—	+·82
Nov.	—	—	—	+·85
Dec.	—	—	—	—

	S. minima against		
	M. hirticollis	*P. severini*	*P. alternata*
Jan.	—	—	+·58
Feb.	—	—	+·56
Mar.	—	—	+·56
Apr.	—	—	+·56
May	+·53	−·73	+·58
June	+·62	−·75	+·51
July	+·78	—	—
Aug.	+·83	+·63	—
Sept.	+·91	+·70	—
Oct.	+·90	+·86	—
Nov.	+·88	+·72	—
Dec.	—	+·84	—

There are no indications of significant relationships between *M. hirticollis*, *P. severini*, *P. alternata* except that for the whole year the two last give −·70 for $n=5$.

There is an indication that the combined influence of abundant chironomids may reduce *P. alternata*

in the early part of the year but there is no way of showing this statistically. This may also be possible in *P. severini* which, in 1941, failed to maintain numbers after April against abounding chironomids. A much higher aggregate would be expected after the March index of 524 (cf. conditions in 1935 and 1936). If this year is excluded as exceptional in this regard and the *P. severini* aggregates for the remaining 7 years are correlated with successive monthly temperatures as before, the coefficients run: Dec. —, Jan. −·77, Feb. −·67, Mar. —, Apr. −·73, May −·61, and June −·64. This series seems to bring out more strikingly the benefits of low winter and spring temperatures to this fly.

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(*Received* 20 *May* 1942)

A comparative account of the life cycles of *Lumbricillus lineatus* Mull. and *Enchytraeus albidus* Henle in relation to temperature

By T. B. Reynoldson, B.Sc., Ph.D.
University of Leeds (now at the Huddersfield Corporation Sewage Works)

(With 3 Text-figures)

Compared with *Lumbricillus lineatus* the cocoons of *Enchytraeus albidus* are smaller and contain fewer eggs whilst the egg production per worm is higher and sterility among the eggs lower. The fecundity of *E. albidus* is greater over a temperature range 1–25° and is not so adversely affected at the higher or lower temperatures as *L. lineatus*. Incubation in *E. albidus* is longer than in *L. lineatus* but the time required for maturation is much shorter, the periods depending upon the temperature. There is a similar relation to temperature as in the case of fecundity, with *E. albidus* again more tolerant to heat and cold. There is no relation between size and temperature but unlike *L. lineatus*, growth occurs after maturation in *E. albidus* and its cocoons (after the first) and their egg content are larger. There is no apparent relation between the setae length and temperature. The temperature relations favour a more rapid increase of *E. albidus* in the bacteria beds than of *L. lineatus* but they are well suited to the distribution of the species under natural conditions, *E. albidus* favouring a terrestrial habitat whereas *L. lineatus* exists in moister places. Further, they show specific physiological differences distinguishing the two species of worms.

In the course of work dealing with the comparative ecology of the two cosmopolitan enchytraeid worms, *Enchytraeus albidus* Henle and *Lumbricillus lineatus* Mull., in relation to the bacteria bed habitat, it became necessary to learn their fecundity and reproductive rates over a temperature range of approximately 0–25° C. This formed a well-defined phase of the work which is conveniently presented as a separate paper. Not only are the results of interest in relation to the above point but they are also of

general ecological interest, since they compare the thermal ecology of two of the most widely distributed and best known types of enchytraeid which are often found living in the same niche (Moszynski, 1930). Furthermore, the literature indicates that our knowledge of the length of life cycles in the Oligochaeta in general is very imperfect and of little real value in the absence of more precise experimental data. Jacot (1940) emphasized the lack of information on this subject with particular reference to the enchytraeid fauna of the forest complex. Lastly, since this type of experiment takes some considerable time to complete and necessitates daily supervision, it is unlikely to be repeated frequently, and so opportunity has been taken to observe the size-temperature relations of these two species for comparison with results obtained in the more quickly breeding insects.

Material and methods

A part of the work dealing with fecundity in *L. lineatus* carried out in 1936–8 has been published (Reynoldson, 1939*a*, *b*), and the methods used then have been repeated in examining fecundity in *E. albidus*. Additional experiments have been done with *L. lineatus* to complete the temperature series, the particular temperatures being approximately 1, 5, 7·5, 10, 15, 20 and 25° C., which fully cover the range normally encountered in the bacteria bed. Both sets of experiments were done during the peak of the breeding season which is coincident in the two species and extends from about November to March. For the purpose of eliminating seasonal variation in breeding intensity in the course of the experiments, each batch of worms collected from the beds was spread equally over the whole temperature range. Only fully sexually mature worms were used in the tests. Much larger numbers of *L. lineatus* have been used because records were kept in experiments not designed specifically for this purpose. With *E. albidus*, thirty worms at each temperature were considered to give a fair sample. The consistency of the results indicates that they are reliable, and further support is given in the case of *L. lineatus* since a control for the 25° C. experiment, which was kept at 15° C., gave results closely agreeing with those previously obtained for this temperature (Table 1).

The cocoons obtained in assessing fecundity were used in the determination of the incubation period and the life cycle was carried on until maturation of the worm was completed by the laying of the first cocoon. The young worms used in the determination of the maturation period were kept in tubes 1 × 1½ in. on a pad of moist cotton-wool, five worms in a tube. When they had reached approximately half-size they were transferred to larger tubes 1 × 2½ in. Their progress was observed on alternate days at which time they were fed on small pieces of scalded *Phormidium* and the sewage used to moisten the wool changed by pipette. When the reproductive organs began to mature the worms were paired off into fresh tubes and observed daily for cocoon deposition. It was generally possible to determine which worm had produced the cocoon by making careful note of the abundance and appearance of the ova in each. This method for finding the maturation period was tested by repeating the experiment for *E. albidus* at 15 and 20° C. and gave the following results:

At 15° C.:	(*a*)	26·6, ±0·73 days;
	(*b*)	25·4, ±0·91 days.
At 20° C.:	(*a*)	28·4, ±0·73 days;
	(*b*)	25·9, ±1·01 days.

The maturation periods for the respective temperatures are not significantly different.

On the same day that a worm deposited a cocoon it was removed from the tube and chloroformed under water, allowed to relax and its length measured to the nearest 0·25 mm. Then it was placed on a slide and, by gentle pressure on the cover-slip placed over it, the contents were squeezed out of the cuticle leaving this sufficiently transparent to measure the attached setae. Ten setae from each worm were measured with a micrometer eyepiece and ten worms were examined at each temperature whenever possible. To eliminate variations of setae length along the worm, three were measured from the anterior end, four from the middle region and three from the posterior end. In addition, since there is some variation in the length of the setae from the outer to the inner side of a bundle and between ventral and lateral bundles, particularly in *L. lineatus* (Stephenson, 1911), the innermost setae of the ventral bundles were always measured. The cocoons deposited were also measured with the micrometer eyepiece for length and breadth, and size is expressed as the area of a median longitudinal cross-section. The number of eggs contained was noted.

Fecundity

The cocoons of *E. albidus* are smaller and more rounded than those of *L. lineatus*, averaging 0·59 × 0·55 mm. compared with 1·18 × 0·84 mm. The number of eggs contained is also generally less, averaging four to five in a cocoon as against six to seven for *L. lineatus*. The fecundity of the two species in relation to temperature is shown in Table 1 and Fig. 1: over the whole range, the fecundity of *E. albidus* is decidedly the greater. Not only is total egg production per worm higher but there is a consistently lower degree of sterility among the eggs of *E. albidus*.

In comparing the effects of temperature on egg production, *E. albidus* is not so adversely affected either by the lower or higher temperatures. This worm is able to maintain a fairly high rate of egg production at a temperature of 5° C., whereas with *L. lineatus* the rate falls off rapidly at 7° C. This contrast in the two species is even more marked at the higher temperatures where egg sterility plays a

more prominent part. With *L. lineatus*, fertile egg production begins to decrease above 15° C. but with *E. albidus* continues to increase up to 20° C., and even at 25° C. fertile eggs are produced in some numbers. At this latter temperature *L. lineatus* was unable to deposit fertile eggs.

The possibility of self-fertilization and parthenogenesis has been shown to exist in *E. albidus* (Dr L. Cernosvitov in private communication; Gavislov, 1935). No such evidence has been obtained for bed, all methods of fertilization and development were possible and, while such points are of considerable interest, they would not seem to affect results from an ecological standpoint in this case.

Development

Incubation. The shorter incubation period of *L. lineatus* is the most interesting feature in this phase of the life cycle, more particularly when considered in relation to the complete cycle which is considerably longer in *L. lineatus*. Generally speaking the period passed in the cocoon by *L. lineatus* is only two-thirds to a half that passed by *E. albidus*. This is an important point in relation to the ecology of these species and will be dealt with more fully in a later communication. This stage of the life cycle exhibits a similar relation to temperature to that demonstrated for fecundity, since *E. albidus* is again the more tolerant of heat and cold. Development can proceed in this species at all temperatures from 1 to 25° C., whereas *L. lineatus* is virtually unable to develop below 5° C. and proceeds with difficulty at 25° C. Because of the inability of *L. lineatus* to deposit fertile eggs at 25° C., some obtained at 15° C. were immediately transferred to 25° C. and observed. Only fourteen out of fifty-four eggs developed normally and hatching took place in 6 days. These eggs were contained in six cocoons, and those able to develop were so distributed that hatching occurred in three of the cocoons. Development in *E. albidus* is greatly retarded below 5° C. and at 1–1·5° C. incubation occupies about 20 weeks. In this species progress is not significantly accelerated by the rise from 20 to 25° C., but in all other cases for both species, acceleration (or inhibition) is significantly different between the successive temperature gradients. Failure to hatch from the cocoon in these tests was not pronounced, and the highest recorded was four out of twenty cocoons at 4·4° C. with *L. lineatus* and three out of forty-seven with *E. albidus* at 20° C.

Table 1. *The fecundity of* E. albidus *and* L. lineatus *in relation to temperature*

	Temp. ° C.	No. worms used	No. cocoons produced	Total eggs per worm	Fertile eggs per worm	% eggs fertile
E. albidus	1–1·5	30	5	0·6	0·2	26·3
L. lineatus	1–1·5	150	0	0	0	—
E. albidus	4·2	30	20	3·6	2·8	79·4
L. lineatus	4·5	160	17	0·7	0·5	68·5
E. albidus	7·0	30	23	3·7	3·2	85·9
L. lineatus	7·4	149	57	2·3	1·6	71·0
E. albidus	9·8	30	26	4·2	3·4	80·7
L. lineatus	9·8	106	54	3·1	2·3	75·6
E. albidus	14·8	30	43	6·2	5·1	83·2
L. lineatus	15·0	128	86	4·7	3·5	73·9
L. lineatus (control)	15·0	15	9	4·7	4·0	84·0
E. albidus	19·6	30	55	9·0	7·8	86·8
L. lineatus	20·2	158	106	4·7	2·7	57·9
E. albidus	24·8	30	25	3·7	1·3	35·1
L. lineatus	25·5	60	1	0·1	0	0

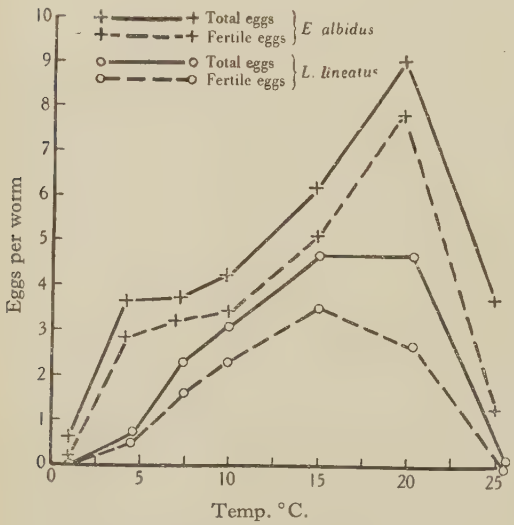

Fig. 1. The fecundity of *E. albidus* and *L. lineatus* in relation to temperature.

L. lineatus, but Gavislov (1935) found that this occurs more generally in the Oligochaeta than had been thought. However, since two worms were contained in each tube in these experiments in addition to the fact that they were collected from the bacteria

Maturation and complete life cycle. In contrast to the incubation period, the maturation period is considerably longer in *L. lineatus* occupying 51–173 days compared with 19–77 for *E. albidus*, depending on the temperature (Table 3, Fig. 3). Since this stage constitutes the greater part of the life cycle, it makes this about 1½–2 times longer for *L. lineatus*.

TABLE 2. *The incubation period of* E. albidus *and* L. lineatus

	Temp. ° C.	No. cocoons observed	Incubation period days	Cocoons failing to hatch
E. albidus	1–1·5	3	137·7	0
L. lineatus	Cocoons from 7° C. used: 1 hatched out of 20 observed			
E. albidus	4·7	16	49·4 ± 1·13	0
L. lineatus	4·4	20	33·2 ± 1·51	4
E. albidus	7·3	19	33·7 ± 0·84	0
L. lineatus	7·5	24	22·7 ± 0·69	2
E. albidus	10·3	20	23·8 ± 1·72	0
L. lineatus	9·8	23	15·7 ± 0·87	0
E. albidus	15·0	38	13·9 ± 0·33	1
L. lineatus	15·5	29	9·9 ± 0·17	0
E. albidus	19·6	47	10·3 ± 0·19	3
L. lineatus	20·3	28	5·6 ± 0·21	0
E. albidus	25·0	9	9·2 ± 0·70	0
L. lineatus	25·0	See text (p. 62)		

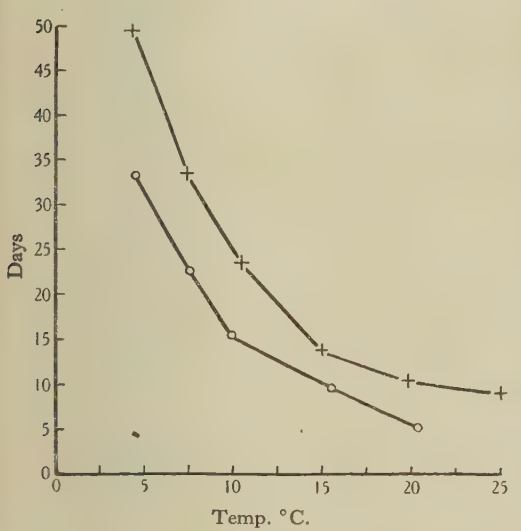

Fig. 2. The incubation period of *E. albidus* +——+ and *L. lineatus* ○——○ in relation to temperature.

The tolerance of temperature already found for these two species with regard to fecundity and incubation is further demonstrated in observations on maturation. *E. albidus* is able to reach maturity at all temperatures from 5 to 25° C., but *L. lineatus* is unable to become mature at 25° C. Twenty-four worms of this species were kept at 25° C. for periods varying from 60 to 180 days without cocoon production, the only sign of approaching maturity being the development of male funnels in two individuals, and these later disappeared without further development. The worms also grew very slowly and never reached the normal mature size. In this species it

TABLE 3. *The length of the maturation period and of the complete life cycle of* E. albidus *and* L. lineatus

	Temp. ° C.	No. worms reared	Maturation period days	Total life cycle days
E. albidus	1–1·5	Five worms kept for 6 months without attaining maturity		
L. lineatus	1–1·5	—	—	—
E. albidus	4·5	20	76·5 ± 2·31	125·9
L. lineatus	4·6	4	172·5 ± 10·22	205·7
E. albidus	7·3	20	62·2 ± 3·29	95·9
L. lineatus	7·5	10	147·0 ± 10·85	169·7
E. albidus	9·9	20	44·5 ± 0·95	68·3
L. lineatus	10·0	16	101·3 ± 6·38	117·0
E. albidus	14·6	20	26·6 ± 0·73	40·5
L. lineatus	14·7	20	62·3 ± 1·40	72·2
E. albidus	20·0	20	28·4 ± 0·73	38·7
L. lineatus	19·6	14	50·5 ± 2·58	56·1
E. albidus	24·6	18	18·6 ± 0·67	27·8
L. lineatus	See text (p. 63)			

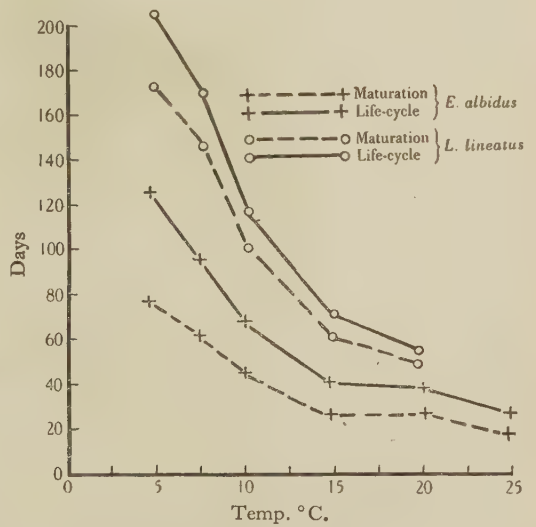

Fig. 3. The maturation period and total life cycles of *E. albidus* and *L. lineatus* in relation to temperature.

was found that the increase in temperature from 4·6 to 7·5° C. did not shorten the maturation period sufficiently to make the difference statistically significant.

From a consideration of the complete life cycle, it is apparent that under the experimental conditions,

multiplication can proceed with *E. albidus* from a temperature of about 5–25° C. and with *L. lineatus* from 7 to 20° C. Over this range, *E. albidus* will have a definite advantage as far as rate of increase is concerned by virtue of the higher fecundity and shorter life cycle. At 10° C., which may be taken as an average bacteria bed temperature for the year, the fecundity of *E. albidus* is approximately one and a half times greater than that of *L. lineatus*, and the life cycle occupies half the time.

The progress-temperature law as originally devised by Krogh, and Sanderson & Peairs (see Chapman, 1931 *b*), permits the rapid calculation of the length of various stages in the life cycle of insects when the time-temperature factors are known and has been applied mainly to this group of organisms because of their economic importance. It is of interest to see how the developmental rates of these enchytraeids fit this law. The thresholds of development and the thermal constants have been calculated from the results obtained at 10 and 15° C. which lie within the favourable temperature zone and are as follows:

Incubation:	*E. albidus*	3·7° C.	157·1	day degrees
	L. lineatus	2·3° C.	118·0	,,
Maturation:	*E. albidus*	2·9° C.	311·5	,,
	L. lineatus	2·5° C.	759·8	,,
Life cycle:	*E. albidus*	3·1° C.	468·6	,,
	L. lineatus	2·4° C.	889·2	,,

Since the thresholds for the different stages are so close together the length of the life cycle at all temperatures is reflected almost directly in the thermal constants and illustrates concisely what has been stated. Further, when the thermal constants are calculated for the other temperatures they approximate to the findings for organisms in general (Chapman, 1931 *a*, *b*). Over the range 7–20° C. for *E. albidus* and 7–15° C. for *L. lineatus* with one exception mentioned below, they exhibit a degree of constancy for all stages in the life cycle. But below 7° C. progress is more rapid than theory demands and development can proceed below the threshold temperatures for both species but particularly *E. albidus*. The non-acceleration of development from 15 to 20° C. in *E. albidus* upsets somewhat the application of the law, and this is also reflected in the total life cycle.

The habitat of *E. albidus* inclines more to the terrestrial type, although it can live in wet places such as the seashore, whereas *L. lineatus* is very rarely found in soil and favours the moister environments (Moszynski, 1930). The temperature relations of these two species are in agreement with this distribution, since the former would have to tolerate a slightly wider temperature range in nature than the latter.

It is recognized that, particularly in the experiments on fecundity, the worms were taken from their normal habitat and transferred to different temperatures without that period of acclimatization normally occurring in nature. But from a consideration of the length of the incubation period and maturation stages it seems likely that for the complete life cycle the results are applicable to field conditions. A further point is that the worms were reared at very nearly constant temperatures, whereas in the bacteria bed there would be some degree of diurnal and daily fluctuation. Experiments comparing the effects of constant and alternating temperatures on rate of development were discussed by Chapman (1931) and Imms (1931), and Buchanan (1940) published data on this subject for Amphibia. It is established that when organisms are subjected to alternating temperatures an acceleration of development is induced, but normally this is not considerable, particularly for such fluctuations as would be experienced in the bacteria bed where a dampening effect is the rule. Lloyd (1935) showed that the diurnal fluctuations in this habitat are considerably reduced and approximate to 5° C. This would not seem to be sufficient to affect the conclusions arrived at regarding the comparative rates of development of *E. albidus* and *L. lineatus*, even if their reactions to this factor were diverse.

Size relations

Worm length. Table 4 shows that there is no consistent relation between size and temperature such as has been demonstrated for insects (Bodenheimer, 1938; Golightly, 1940). Some of the results for the different temperatures are significantly different from others while some are not, but any general trend is lacking. Sizes are also given in this table for sexually mature worms trapped in the beds, and the interesting point emerges that there is a decided difference in the size at which maturation occurs for these two species. For example, the average size at maturation of laboratory-bred *E. albidus* over the whole temperature range is markedly smaller (statistically significant) than the average size for the natural population, suggesting that for this worm considerable growth takes place after maturation. The figures are 6·25 mm. ± 0·24 (at 7° C.) for laboratory-bred worms and 9·2 mm. ± 0·056 for the field population. The sizes of the two groups overlap, indicating that the field collections contained some worms which had recently attained maturity. In contrast, with *L. lineatus* it was found that laboratory-bred worms were slightly but significantly larger (15·2 mm. ± 0·30) than those of the field population (14·1 mm. ± 0·16), suggesting that little or no growth takes place after sexual maturity is reached. This slight difference might be due to the fact that under laboratory conditions abundant rich food was available all the time, whereas, in the bacteria beds, periods of acute food scarcity occur from time to time (Lloyd *et al.* 1940; Reynoldson, 1939 *a*, *b*). Lloyd pointed out that the evidence of different specific behaviour is not conclusive, basing his argument on the fact that Go-

lightly (1940) showed that laboratory-bred *Psychoda* is smaller than the field population when both are reared at the same temperature. But if such a case is put forward for *E. albidus*, it cannot be maintained for *L. lineatus* and no other explanation seems available to account for the overlapping in size between the laboratory-reared worms and the field population in the former species. Golightly suggested some deficiency in the food of his laboratory-reared flies to account for their smaller size, but this again cannot account for the difference in behaviour of the two enchytraeids. The matter could be decided by rearing both species, measuring worm size on deposition of the first cocoon, and then observing if growth took place subsequently. Unfortunately, it was not possible to do this.

Setae length. No relation is apparent with *E. albidus* between setae size and the temperature at which the worms are reared, thus agreeing with the results

TABLE 4. *The length of the adult worm reared at the different temperatures and of the field population*

	Temp. ° C.	No. worms measured	Size of worms mm.	Range of size mm.
E. albidus	4·5	20	5·84 ± 0·44	
	7·3	16	6·25 ± 0·24	
	9·9	19	5·74 ± 0·16	
	14·6	19	6·08 ± 0·13	4·75– 7·75
	20·0	20	5·47 ± 0·09	
	24·6	16	5·66 ± 0·14	
Field population		50	9·20 ± 0·06	7·0 –12·0
L. lineatus	4·6	3	14·3 —	
	7·5	9	15·6 ± 0·41	
	10·0	15	17·1 ± 0·35	8·5 –19·5
	14·7	15	14·1 ± 0·59	
	19·6	13	14·1 ± 0·62	
		Av.	15·2 ± 0·30	
Field population		40	14·1 ± 0·16	12·0 –16·0

for worm length. The fact that there is no correspondence between variation in worm length and setae length at the different temperatures for this species is probably due to the small variation in average size of the worm which, in the whole series, only amounted to 0·78 mm. (Table 4). The difference between the setae length of laboratory-reared worms (12·7 ± 0·12 units) is, however, markedly different from those of mature worms collected in the field (14·2 ± 0·19 units). This is obviously related to and emphasizes the similar finding in worm length. The experiments with *L. lineatus* are not quite so complete, and the results at 10, 15, and 20° C. might indicate a tendency for decrease in size with increase in temperature but, as the results are not statistically significant between 10 and 15 and 15 and 20° C. this cannot have great weight, particularly in the absence of confirmatory findings in other measurements. The setae size of the laboratory worms is not significantly different from those of mature specimens of the natural bed population, the figures being 22·7 units and 23·4 ± 0·48 units respectively (Table 5). This is no doubt the result of the small differences found for worm length, and indicates further, that little or no growth takes place after sexual maturity is reached.

TABLE 5. *The length of setae of adult worms reared in the laboratory and of the field population.* (1 unit = 0·04 mm.)

	Temp. ° C.	No. worms measured	Length of setae
E. albidus	4·5	10	12·8 ± 0·18
	7·3	10	12·0 ± 0·27
	9·9	10	12·3 ± 0·16
	14·6	10	13·3 ± 0·22
	20·0	10	13·7 ± 0·26
	24·6	10	12·2 ± 0·23
		Av.	12·7 ± 0·12
Field population		10	14·2 ± 0·19
L. lineatus	4·6	3	22·6 —
	7·3	7	23·1 —
	10·0	10	23·2 ± 0·18
	14·7	10	22·8 ± 0·39
	19·6	10	21·6 ± 0·44
		Av.	22·7 ± 0·33
Field population		10	23·4 ± 0·48

Cocoon size and egg numbers (Table 6). The sizes of the first cocoons and the egg contents deposited by the laboratory-bred worms were contrasted with those obtained from worms collected in the field and in addition, in the case of *E. albidus*, with those deposited in the field. The results follow the same trend as those noted for worm and setae length. With *E. albidus* the cocoons deposited by the laboratory-reared worms are not only significantly smaller but contain less eggs than either those obtained from collected worms or those cocoons deposited in the beds. Also, the cocoon sizes of these latter two groups were not significantly different, but cocoons collected in the field contained a slightly smaller number of eggs. This is probably due to the sampling, since only mature worms of normal size were chosen for obtaining cocoons, the smaller more recently mature worms being neglected.

Cocoon size and egg numbers in *L. lineatus* tend to emphasize more strongly the slightly larger size of the laboratory-reared worms. The cocoons obtained from the latter are significantly larger than those deposited by worms from field collections. This contrast is marked in respect of the number of eggs contained in the cocoon: in the laboratory-reared individuals the number was 10·3 ± 0·5 and in the other class 6·3 ± 0·36. The explanation already given for worm length is also applicable here; it is probably emphasized for egg numbers because worms from the field may have recently deposited cocoons, thus reducing the egg content of subsequent cocoons, whereas, in the laboratory, it was the first cocoon

deposited by a fully mature individual. This contrasts strongly with the findings for *E. albidus* where the first cocoons have a much smaller egg content which, in later cocoons, rapidly increases until the normal egg content of 4–5 per cocoon is reached.

TABLE 6. *Cocoon size (in sq. mm.) and the number of eggs contained*

	First cocoon	Normal cocoon	Field cocoon
E. albidus	0·17 ± 0·001 (50 measured)	0·33 ± 0·002 (25 measured)	0·33 ± 0·002 (25 measured)
L. lineatus	1·36 ± 0·026 (47 measured)	0·97 ± 0·031 (50 measured)	—

Egg numbers per cocoon

	First cocoon	Normal cocoon	Field cocoon
E. albidus	2·1 ± 0·11 (50 cocoons)	5·5 ± 0·28 (25 cocoons)	4·1 ± 0·26 (25 cocoons)
L. lineatus	10·3 ± 0·50 (47 cocoons)	6·3 ± 0·36 (50 cocoons)	—

DISCUSSION

The results show very distinct differences in the influence of temperature upon the two species of enchytraeid worms, *E. albidus* and *L. lineatus*, which must exert a profound effect upon their distribution and their capacity to maintain themselves in a particular environment quite apart from their ability to compete effectively with other organisms.

E. albidus can tolerate a wider range of temperature than *L. lineatus* at all stages and does not suffer so adversely at high and low temperatures. In spite of smaller cocoons with fewer eggs per cocoon, *E. albidus* has greater fecundity over the whole temperature range, whilst the percentage sterility is less. Again, although the incubation period is longer these worms mature more rapidly, the total time being graded with the temperature which is a factor in the environment, therefore, more favourable to the increase of *E. albidus* than *L. lineatus*. Even within the temperature limits of the bacteria beds the former has a decisive advantage and its absence from some beds must be determined by some other factor or conditions yet to be revealed (Reynoldson, 1939 *a*, *b*). On the other hand, the wide tolerance of *E. albidus* is well suited to the terrestrial habitat, which is more characteristic of this species in its distribution under natural conditions, in contrast to the narrower limits of moist environments preferred by *L. lineatus*.

These temperature relations coupled with the growth after maturity in *E. albidus* and reflected in the increase in size of their cocoons—features lacking in *L. lineatus*—provide specific physiological differences serving to distinguish still further these two species of enchytraeid worms.

The writer is indebted to the Department of Scientific and Industrial Research for a Senior Research Scholarship by which this work was entirely financed. He also wishes to thank Prof. E. A. Spaul and Dr Ll. Lloyd of the Zoology Department, University of Leeds, for their encouragement and help in the course of the work and for their useful criticism of the manuscript. It is a pleasure to acknowledge the kindness of Dr L. Černosvitov, formerly of the Prague Museum, for confirming identification of the worms and also for supplying unpublished data and certain references. Finally, the author wishes to thank Dr H. H. Goldthorpe and Dr J. Nixon of the Huddersfield Corporation Sewage Works Staff and Mr J. T. Thompson of the Leeds Corporation Sewage Works Staff for their active co-operation in the field work.

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(*Received* 1 *May* 1942)

The resistance of potato varieties to *Heterodera schachtii* Schmidt, the potato-root eelworm

By Alan R. Gemmell, Ph.D.
Botany Department, University of Glasgow

The potato varieties Epicure and Doon Star permitted the production of fewer and smaller cysts of *Heterodera schachtii* on their roots than the varieties Golden Wonder and Majestic, when all were grown under standard conditions. Under the stimulus of potato-root excretion the number of larvae which emerged from Epicure and Doon Star cysts was less than that from Majestic or Golden Wonder cysts of the same volume. Accepting Lapage's (1938) criteria of resistance to nematode infestation in animals and applying them to plants, it is concluded that this deleterious effect of the variety on the eelworm is probably of the nature of a degree of resistance to eelworm attack specific to each variety and is physiological and not anatomical in action. Of the varieties tested Epicure is judged the most resistant, closely followed by Doon Star. Majestic is fairly susceptible but not so much so as Golden Wonder, on which the eelworm forms large cysts that are extremely productive of larvae.

The resistance of potato varieties to the potato-root eelworm has been little studied, and only two papers deal with British varieties (Robertson, 1939; O'Brien & Prentice, 1931). The reason for this lack of information on an important subject is not clear, but the difficulty of obtaining adequate controls suggests itself. Robertson (1939) studied the effect of an increasing eelworm infestation on the yield of several varieties, and his results indicated that Epicure was the most resistant. O'Brien & Prentice (1931) followed a similar procedure and issued a list of varieties in which Epicure is classed as extremely susceptible.

It is essential to determine exactly what is meant by resistance to *Heterodera schachtii*. A variety yielding highly in infested soil might not be resistant but merely tolerant, the nematode being able to complete its life cycle and multiply without much difficulty. The yield, too, of a so-called resistant variety might only be a reflexion of the ability of the plant to replace the roots damaged by the eelworm and so suffer no appreciable loss. To farmers the question of yield must always be important, but if in obtaining that yield a very large eelworm infestation is built up in the soil through the use of a tolerant variety, the stage will be reached ultimately when even the most tolerant variety will be unable to maintain itself and the growing of potatoes must cease.

In making an estimate, therefore, of the degree of resistance of a potato variety to *H. schachtii* the effect of the variety on the nematode must also be considered. Thus, it is possible that a variety may suffer from eelworm infestation and have its tuber production much reduced, but if the nematode could not form new cysts on its roots or only small poorly developed cysts then the soil infestation would decrease and the growing of potatoes become more, instead of less, economical with continued cropping. Lapage (1938) cites the following as criteria of resistance to eelworm infection:

'(1) Failure of the nematode to live inside the host or its early death in it.

'(2) Decrease in its production of eggs or larvae.

'(3) Inhibition of its growth or development...so that the adults require longer to mature or do not live so long when they are mature, sometimes being stunted or smaller.'

These criteria can be applied to plant parasitic nematodes, and it is from this standpoint that the present investigation was made.

Material and methods

The experiments were not intended to be indicative of the yields of the varieties under field conditions but rather, by using a controlled environment, to determine the effect of a standard rate of invasion on the plants and on the number and kind of cysts which they would produce. Setts were planted in pots each containing 20 lb. of sterile sand plus artificial fertilizer (I.C.I. no. 2) at the rate of 10 cwt./acre, and received a weekly quota of freshly hatched eelworm larvae until 20,000 had been added to each pot. Each variety was replicated five times with five uninfested pots as controls, the latter receiving quantities of root excretion similar to those containing the larvae added to the other pots.

Tubers were planted on 27 May 1940, in pots randomly distributed in the greenhouse. The plants were allowed to come to full maturity and die. In counting the cysts the five pots comprising each series were tipped out and the sand mixed, after which 50 g. samples were used for each count. Care was taken that all the cysts adhering to the roots were scraped off and included in the bulk soil sample. The counts were done by the Morgan flotation method, and as it was found that the control pots contained no cysts the accuracy of this method

for determining the number of cysts added to the soil by each variety was considered satisfactory. The counting of larval emergence was done using the single-cyst technique (Gemmell, 1940), and the measurements of cyst size were made by means of a standardized eyepiece micrometer.

Results

The yield of tubers in pot tests is variable and no conclusions will be based thereon, nor will the actual yielding powers of the varieties be considered. The results of the cyst counts are given in Table 1. The

Table 1. *Number and size of cysts per* 50 *g. sand borne by four potato varieties*

Variety	No. of cysts/50 g.*	Mean diam. of cysts† mm.
Epicure	2·6 ± 0·42	0·488 ± 0·01
Doon Star	3·2 ± 0·4	0·472 ± 0·01
Majestic	4·9 ± 0·43	0·512 ± 0·01
Golden Wonder	6·3 ± 0·68	0·528 ± 0·01

* Av. of 20 counts.

† Av. of 250 measurements and is equal to ½ (length + breadth).

solve the problem whether these differences are caused by true resistance or are expressions of the length of life of the host. There is no doubt that earliness or early lifting of a crop has a marked effect on cyst size which is independent of the variety of potato. This has been shown to exist in the field where, although conditions may vary slightly in different districts, a series in cyst size can be demonstrated which correlates with the date of lifting of the crop. However, for reasons which will be advanced later, the earliness of any specific variety is not considered sufficient cause for the variation in cyst size noted above.

To study the different types further, an examination of the emergence of larvae from random samples of cysts of each variety was made, using fifty cysts as a sample and employing the single-cyst technique. This experiment was repeated three times at different seasons of the year, the cysts stored after each trial, and an attempt made to obtain further emergence after some time had elapsed. The same sample of potato-root excretion was used in all the experiments to induce larval emergence, and the plates containing the cysts were stored in an incubator at 20–21° C. The results of these experiments are summarized in Table 2.

Table 2. *The emergence of larvae from cysts borne on different potato varieties*

	Exp. 1 10. x. 40–19. iii. 41		Exp. 2 27. i. 40–3. iii. 41		Exp. 3 11. iii. 41–2. vi. 41	
Variety	% cysts hatching	Larvae per cyst	% cysts hatching	Larvae per cyst	% cysts hatching	Larvae per cyst
Epicure	80	70 ± 9·5	76	93 ± 15	84	117 ± 12
Doon Star	84	106 ± 10	64	124 ± 19	76	125 ± 13
Majestic	80	142 ± 18	92	179 ± 15	94	163 ± 14
Golden Wonder	92	159 ± 14	90	231 ± 21	100	276 ± 17

potato varieties are arranged here roughly in the order of maturity, i.e. Epicure is a first early, Golden Wonder a late variety, and the other two are intermediate. There is a distinct series starting from the lowest cyst count and the earliest potato, Epicure, through the second earlies to the maincrop and the highest cyst count, Golden Wonder.

There are several possible reasons for this seriation. There might be fewer roots on the earliest variety with the consequent lessening of the chances of infestation by the larvae, or, owing to the earliness of the variety a considerable number of the larvae might not be able to complete their life cycle. The reaction, on the other hand, might be the expression of true physiological resistance resulting in the inability of the larvae within the roots to form cysts.

An examination of the cysts from the varieties grown in the pot tests showed that the size of the cyst is also measurably affected by the variety of potato on which it is formed. This variation is not great but is statistically significant (Table 1). The range in size is definite proof of an appreciable effect of variety on the cyst of *H. schachtii*, but it does not

It is clear that the variety of potato affects the output of larvae from cysts borne on it, the extreme instances being Epicure and Golden Wonder. Since the same root excretion was used throughout, the differences in the larval emergence must be due to some factor inherent in the cyst itself, and, since the parent larvae from which all the cysts in question are descended were drawn from a mass sample, the cause of the variation between the cysts must be the potato variety.

It is difficult to account for the relatively high percentage of unproductive cysts in the Epicure and Doon Star collections, and as the latter reaches maturity at about the same time as Majestic, the aborted cysts in this case at least must be a varietal effect having the nature of true physiologic resistance, and not the result of the time available for the nematode to complete its life cycle. The low larval emergence from Epicure and Doon Star cysts could be attributed to the action of this resistance factor and also to the smaller size of the cysts of these varieties. It was shown (Gemmell, 1940) that the number of larvae liberated is proportional to the size

of the cyst and also to the number of eggs which it contains, but this cannot be regarded as the reason for the differences apparent in Table 2.

Each cyst from which larvae were induced to emerge was measured, the volume of the cyst and the number of larvae which it would produce being compared (Table 3). There is little difference in the number of larvae emerging from cysts of the maximum size of any variety, but there is evidence in all the other groups that Majestic and Golden Wonder cysts produce more larvae than cysts on the other varieties. Measurements of larvae from cysts borne on the different varieties has shown that the larvae of the more productive varieties are not smaller than those of the less productive ones, and the only factor to which the differences demonstrated can be attributed is one dependent on the variety of host, for, so far as possible, all other variables were eliminated from the experiments.

TABLE 3. *The relationship between volume and number of larvae produced by cysts grown on four different potato varieties*

	Av. no. of larvae from cyst of volume (c.mm.)				
Variety	0·208–0·137	0·137–0·100	0·100–0·058	0·058–0·037	0·037–0·017
Epicure	220	111	100	69	53
Doon Star	229	141	100	131	73
Majestic	209	168	171	141	126
Golden Wonder	236	268	214	125	115

DISCUSSION

The results demonstrate a positive effect of the variety of potato on the cysts of *H. schachtii* formed on its roots. This influence reflects the reaction of the host variety to nematode invasion, and if it hinders the establishment of the nematode or limits its potential reproductive capacity, it can be said that the variety is exhibiting resistance. In certain instances, plants react to invading organisms in a manner similar to that exhibited by animals; thus it is possible to immunize a potato plant against certain virus diseases by inoculation with very mild virus. By the application of Lapage's criteria of resistance to nematode infestation in animals to the results obtained with the potato a number of points of resemblance are at once apparent.

(1) *Failure of the nematode to live inside the host or its early death in it.* As no extensive examination of the nematode within the host was made in the experiments no direct evidence can be cited indicating the failure of *H. schachtii* to live inside the roots of the potato plant. The results of the cyst counts, however, bear directly on this aspect of disease resistance. The initial infestation of the pots with larvae was secured by using aliquot portions of a larval suspension; it is therefore reasonable to assume that equal numbers of larvae were added to each pot. The variation in number due to the chance inclusion of a few extra larvae in some pots should be eliminated by the number of replications of each variety tested. The production of equal numbers of cysts by each variety might therefore have been expected, but in fact only one-third as many cysts were produced by Epicure as by Golden Wonder. Possibly the latter had a greater root development than the former, increasing the chances of infestation, but there is equally the possibility that Epicure was exhibiting a form of resistance of the type indicated by Lapage's first criterion and a number of larvae either could not establish themselves within the host or else died prematurely. If one assumes this latter view to be correct then Epicure and Doon Star may be said to exhibit true resistance to nematode infestation.

(2) *Decrease in the production of eggs or larvae by the nematode.* This, the second of the criteria of resistance to nematode infestation, is markedly exemplified by the results described. The difference in production of larvae by cysts of the same volume produced on different varieties is pronounced, and once again Epicure gives the most decided reaction indicating resistance to eelworm infestation, namely, a decrease in the number of larvae produced in comparison with Golden Wonder or Majestic. Examination of cysts from the 'resistant' varieties has not indicated any notable reduction in egg content when compared with the susceptible varieties but, in the former case, a large number of the contained eggs are incapable of hatching. The cause of the decrease in larval production seems, therefore, to be in the development of larvae.

Further, in Epicure and Doon Star only about 80% of the cysts are productive even under favourable conditions, while in Majestic and Golden Wonder the percentage rises to nearly 100. Examination of the non-productive cysts has shown that in many instances the eggs contain only disorganized contents, while in other cysts there is no trace of either eggs, larvae or egg cases. This condition is relatively common in the 'resistant' varieties and could be classed as the complete abortion of all the eggs inside the young female cyst. Here again, therefore, is evidence of a high degree of resistance to eelworm infestation in Epicure and Doon Star.

(3) *Inhibition of growth or development of the adult nematode.* This criterion of resistance is a mild expression of that type of reaction instanced in para. (1), for here the larvae survive to become underdeveloped or inhibited adults, whereas in the first case they are killed outright. Nevertheless, examples of this type of resistance can be seen in the varieties studied. The cysts, i.e. the adult females, are definitely smaller on Epicure or Doon Star than comparable cysts on Golden Wonder or Majestic. This decrease in size may be due to the two former varieties being earlier in reaching maturity than Golden Wonder, but there is little if any difference in this respect between Doon Star and Majestic,

and it is hardly likely that the lateness of the variety is the causal factor.

Also, under the experimental conditions the period during which larvae were introduced was limited, but between the last addition and the death of the earliest variety Epicure there was ample time for the full life cycle of the nematode. Another observation bears on the question of the time of development, namely, that the largest Epicure cysts were comparable in size to the biggest Golden Wonder cysts, but the distribution of sizes within the range varied greatly. Thus, if the size of the cyst were solely a function of the length of time spent by the nematode on the host, the maximum size of cyst in the two varieties would differ with the different dates of ripening of the host. Since this is not so, it appears that cysts borne on Epicure have sufficient time to reach their full development, and the fact that the average size is smaller than on Golden Wonder indicates the existence of this effect of plant resistance. It might be argued that the nematode in the roots of Epicure takes longer to mature than in Majestic or Golden Wonder, but this admits the resistance of the former variety, since the time required by the nematode to mature lengthens with the degree of resistance of the host (Lapage, 1938).

From all the above evidence it can be said that Epicure and Doon Star are varieties which have a fair degree of resistance to invasion by *H. schachtii* when compared with Majestic and Golden Wonder. Robertson (1939) was of the opinion that the high yields which he obtained from Epicure were due to the tolerance by the variety of the toxins secreted by the nematode, but from the above results it is clear that this is not the sole contributory factor, as the decreased number of cysts produced per plant and the reduced number of larvae which they contain must affect the amount of food material taken by the nematode from the plant.

Another factor which explains Robertson's results is the slower rate of increase in infestation which will occur in soils in which Epicure has been grown, with the result that the final infestation will be considerably lower. This may be a reason why growers of early potatoes on the Ayrshire coast who confine themselves to Epicure are able to grow potatoes in the same fields for many years even where considerable infestation of *H. schachtii* has been built up, whilst a corresponding condition in Lincolnshire or Yorkshire would preclude the growing of potatoes.

There will necessarily be an increase in the nematode infestation of the soil until the biological equilibrium or the economic limit to the growing of potatoes is reached. This point will come later with Epicure than with Golden Wonder, and it is probable that the equilibrium level of host and parasite will be more in favour of the host in the former case. Further, any factor such as early lifting which tends to upset the host-parasite relationship in favour of the host will still more delay the onset of the time after which potatoes cannot profitably be grown.

The precise action of the resistance mechanism in varieties such as Doon Star and Epicure is at present unknown. Examination of the rootlets has shown that invasion takes place without any apparent difficulty from the anatomical aspect, so that the mechanism is probably physiological in nature and may take the form of an effect of the plant on the nematode comparable to the antibody reaction in animals. It is also possible that resistant varieties are, as Robertson suggested, more tolerant of the toxins secreted by the eelworm in the plant.

From the evidence presented, it is concluded that there is a reaction by the plant to the eelworm attack which is specific to each potato variety and which may take the form of resistance to the unlimited development of the nematode. In the varieties tested this resistance is greatest in Epicure and Doon Star and least in Majestic and Golden Wonder.

This work was done during the tenure of a Carnegie Fellowship at the West of Scotland Agricultural College, and the Botany Department, Glasgow University.

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(*Received* 13 *May* 1942)

The statistical treatment of toxicological data relating to more than one dosage factor

By D. J. Finney, M.A.
Rothamsted Experimental Station, Harpenden, Herts

(With 2 Text-figures)

Experiments on the biological measurement of toxicities, and others requiring the use of the probit transformation in the statistical analysis, have in the past been largely of a unifactorial nature. In order to reach a fuller understanding of the action of insecticides and other poisons it is necessary that experiments be performed in which two or more factors relating to the dosage are subjected to variation. Bliss has recently described methods for the analysis of two-factor experiments, but his technique is not a direct extension of the simple case for the fitting of a probit regression line, nor will it generalize easily when more factors are involved.

In the present paper it is shown that probit regression planes and hyper-planes can be fitted in a way exactly analogous to that used for the regression line. Tests of heterogeneity and of parallelism, and standard errors of parameters are readily derived. The notion of relative potency of two poisons does not extend so easily, and it is suggested that the mean probit difference should be used in its stead as a measure of their relative effectiveness. As in the unifactorial analysis, the routine of fitting the regression equations may be repeated until satisfactory accuracy in the estimation of the parameters is attained, but usually one, or at the most two, cycles of the process gives values sufficiently near to the maximum likelihood solution for practical purposes.

A more general class of equations is introduced for use when the probit mortality is linearly related to each dosage factor separately, but the individual effects are not additive. The suggestion made by Bliss (1940) for this case lacks symmetry and does not completely fulfil the linearity conditions, but the present proposal avoids these defects and permits the making of tests for the significance of the improval in fit effected by the additional parameters.

The arithmetical procedure for the fitting of a probit plane is described in detail for data on the toxicity to *Tribolium castaneum* of a pyrethrin oil spray at various levels of concentration and deposit weight. Two methods of applying the poison were compared in this experiment, and the two series of results were excellently fitted by parallel probit planes. Direct spraying gave slightly, but not significantly, higher kills than the film method of application, and under the particular conditions obtaining, increasing the concentration of pyrethrin in the spray was a far more effective means of increasing kill than was a proportionate increase in the total amount of spray available to the insects. Numerical examples used by Bliss are also discussed in relation to the methods of fitting and forms of equation given in this paper.

One of the most important recent developments in biological experimentation has been the introduction of factorial design amongst the treatments to be investigated. This concept has been particularly useful in the planning of agricultural field trials, but its value in the logical structure and interpretation of experiments is as great in the laboratory as in the field. The contrast between the factorial principle and the older one of 'varying the essential conditions *only one at a time*' has been well expressed by Fisher (1942, § 37): 'We are usually ignorant which, out of innumerable possible factors, may prove ultimately to be the most important, though we may have strong presuppositions that some few of them are particularly worthy of study. We have usually no knowledge that any one factor will exert its effects independently of all others that can be varied, or that its effects are particularly simply related to variations in these other factors. On the contrary, if single factors are chosen for investigation, it is not because we anticipate that the laws of nature can be expressed with any particular simplicity in terms of these variables, but because they are variables which can be controlled or measured with comparative ease. If the investigator, in these circumstances, confines his attention to any single factor we may infer either that he is the unfortunate victim of a doctrinaire theory as to how experimentation should proceed, or that the time, material, or equipment at his disposal are too limited to allow him to give attention to more than one narrow aspect of his problem.'

In that branch of toxicological research which deals with insecticidal problems, attention has been primarily directed to the effects of variation in the concentration of the toxic substance on the mortality rate of the insects. To a lesser extent the time of exposure of the insects to the poison has been studied, though there is comparatively little published data relating to tests in which both these factors have been varied. Other factors such as the temperature, or the quantity of poison employed, have received even less attention. Before the action of an insecticide can be fully understood, the direct effects and the interrelationships of these and other factors must be investigated in detail. The present paper indicates statistical technique appropriate to the analysis of

data from multi-factorial tests, and illustrates the procedure on some of the available numerical material. The arithmetic may at first appear laborious; experience will reduce it to a fairly simple routine, and the adoption of carefully controlled experiments should eventually enable it to be replaced, at least as a reasonable approximation, by more rapid graphical methods.

1. The probit plane

Transformation of the observed percentage mortalities to the probit scale (Gaddum, 1933; Bliss, 1934, 1935*a*) has proved a valuable aid to the analysis of data on series of concentrations of poisons, since the relationship of the probit and the logarithm of the concentration is frequently linear. The procedure for obtaining the maximum likelihood solution for this line is described by Fisher & Yates (1938, Introduction).

Since it has also been found that, for a fixed concentration, the relationship between the mortality probit and the logarithm of the time of exposure is often linear, as a representation of the joint effect of time and concentration it seems reasonable to examine first a plane,

$$Y = a + b_1 x_1 + b_2 x_2,$$

giving the probit in terms of the log-concentration (x_1) and the log-time (x_2). Bliss (1940) expressed this by saying that the time and concentration required to give any particular kill are linearly related. He proposed to obtain the relationship by estimating either the log-concentrations giving a selected kill for a series of times or the log-times giving a selected kill for a series of concentrations, according as the experiment was performed in batches of fixed exposure time and varied concentration or of fixed concentration and varied time. A more symmetrical approach, however, and one that is an immediate generalization of the one-factor analysis, is to apply regression methods to determine the coefficients b_1 and b_2 directly.

In order to do this, it is first necessary to fit a provisional plane to the empirical probits read directly from probit tables. If the experiment has been arranged as a number of concentration tests at each of a series of times, it is possible to use simply lines drawn by eye to fit the probit log-concentration points for each time, with the restrictions that all these lines shall be parallel and at distances apart proportional to the differences in their log-times. The procedure for the alternative arrangement of a number of time tests at each of a series of concentrations is similar. If the structure of the experiment is such that a series of concentrations and a series of times are used in all, or nearly all, their combinations, a method adapted from a suggestion of Richards (1941) may be used. If the probits are plotted against the sum of log-concentration and log-time, $(x_1 + x_2)$, points with constant x_2 should lie on one set of parallel straight lines with slope b_1, and points with constant x_1 should lie on a second set of parallel lines with slope b_2. In this way a plane representation of the three-dimensional figure relating probits to log-concentration and log-time is obtained, and provisional probits can be read from two intersecting sets of parallel lines drawn by eye in this diagram. Whatever the means employed, it is worth taking some trouble over the drawing of the provisional lines, in order that satisfactory accuracy may be obtained in one stage of the fitting.

Values of the working probit, y, and its weight, w, may be derived from the provisional value and the percentage kill in precisely the same way as when only one factor is involved. By the technique of multiple regression, an equation of the form given above may be fitted to the values of y. If this equation differs substantially from the provisional lines, it may be used to determine a new series of provisional probits with which the cycle of computations is then repeated. An example of the computations, together with those necessary for the testing of the goodness of fit and determining of standard errors, follows.

2. The toxicity of a pyrethrum oil spray to *Tribolium castaneum*

As an example of the arithmetical procedure of fitting probit planes, data recently obtained at Rothamsted on the toxicity of a pyrethrum oil spray to *T. castaneum* may be considered. In this experiment the factors under test were the concentration of pyrethrin I in the oil and the weight of spray deposited on the disk on which the insects were placed. All combinations of four concentrations and three deposits were tested, about thirty insects being used in each case. Two techniques were used for applying the poison to the insects. In the first, the 'spray' method, the poison was sprayed directly on to the insects; in the second, the 'film' method, the insects were placed on a disk which had previously been sprayed so as to cover it with a film of poison. Tests made with the oil alone showed no appreciable variation in mortality for either technique over the range of deposits used, and an average figure from these tests was used as a control mortality for adjusting the observed kills, according to the formula

$$p = \frac{p_o - p_c}{100 - p_c},$$

where p_o, p_c, p are respectively the observed, control, and adjusted percentage kills (Tattersfield & Morris, 1924; Abbott, 1925) (see Table 1).

It was desired to represent the relationship between the probits of the kill, log-concentration* (x_1) and log-deposit* (x_2) by a plane for each technique.

* To simplify the computing, the concentrations were doubled and the deposits multiplied by 10; i.e. x_1 and x_2 as used here are respectively 0·30 and 1·00 greater than the log-concentrations and log-deposits obtained from Table 1.

The empirical probits of the percentages shown in Table 1 (excluding 0 and 100% kills) were therefore plotted against $(x_1 + x_2)$ and, for each technique, two intersecting sets of parallel lines drawn to represent the regressions of probits on x_1 for fixed x_2 or x_2 for fixed x_1. The figure was similar to that shown in Fig. 1, which represents the final result of the fitting. From these lines expected probits were read (one decimal figure here, as usual, giving sufficient accuracy) for each pair of values x_1, x_2 (which may conveniently be described as the dosage).

The next stage of the fitting is very similar to that for the one-factor case. Details for the spray method are set out in Table 2 in which successive columns show x_1, x_2, n (the number of insects), p (the percentage kill), the empirical probit of p, and the expected probit. Exactly as described by Fisher & Yates and obtained from their Table XI, weighting coefficients, w, and working probits, y, are calculated. The products wx_1, wx_2, and wy are next formed; for wx_2 it is sufficient to total the values of w for each of the three deposits and multiply this total by the

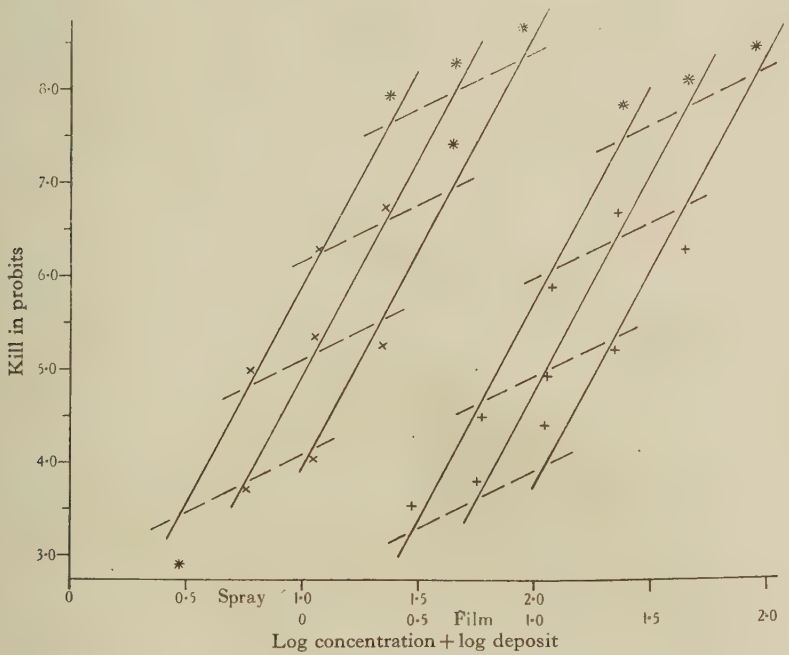

Fig. 1. Toxicity of pyrethrin oil spray to *T. castaneum*. × direct spray technique; + film technique; * 0% or 100% kill; —— effect of change in concentration for fixed deposit; – – – effect of change in deposit for fixed concentration.

TABLE 1. *Percentage kills of* T. castaneum *by a pyrethrum oil spray, adjusted for 3·9% mortality amongst the controls*

(Numbers of insects shown in brackets)

Conc. mg./c.c.	Deposit (mg./sq. cm.) Spray 0·29	Spray 0·57	Spray 1·08	Film 0·29	Film 0·57	Film 1·08
0·5	0·0 (27)	10·3 (29)	16·8 (30)	6·7 (29)	11·4 (27)	25·7 (28)
1·0	49·8 (29)	64·1 (29)	61·0 (24)	30·7 (30)	48·0 (28)	59·1 (28)
2·0	89·6 (30)	96·1 (27)	100·0 (31)	82·1 (29)	96·3 (28)	92·6 (28)
4·0	100·0 (28)	100·0 (30)	100·0 (19)	100·0 (29)	100·0 (29)	100·0 (17)

appropriate x_2, but for wx_1 and wy separate entries must be made for each of the twelve tests and these products totalled in the three deposit groups as shown. Totals of the columns, Sw, Swx, Swx_2, Swy, are then made at the bottom of the table.

By summing the products of each of the twelve values of wx_1 with x_1 and deducting $(Swx_1)^2/Sw$, the sum of squares of deviations of x_1, denoted by Swx_1^2, is obtained. Similarly, multiplying each of the three subtotals of wx_1 by the corresponding x_2, adding, and deducting $(Swx_1)(Swx_2)/Sw$, the sum of products of deviations, Swx_1x_2, is obtained. In like manner all the sums of squares and products shown in Table 3, for both spray and film methods, have been computed. The values for the two techniques are added to give the third column of this table.

Inspection of the figure showing provisional probit

TABLE 2. *Computations for fitting of probit plane for toxicity of pyrethrin oil spray to* T. castaneum *(direct spray technique)*

x_1	x_2	n	p	Empirical probit	Expected probit	w	y	wx_1	wx_2	wy	Y
0	0·47	27	0	$-\infty$	3·4	6·4	2·91	0		18·624	3·44
0·30	0·47	29	49·8	5·00	4·8	18·2	5·00	5·46		91·000	4·84
0·60	0·47	30	89·6	6·26	6·3	10·1	6·26	6·06		63·226	6·25
0·90	0·47	28	100·0	∞	7·6	1·1	7·94	0·99		8·734	7·65
						35·8		12·51	16·826	181·584	
0	0·75	29	10·3	3·74	3·8	10·7	3·74	0		40·018	3·79
0·30	0·75	29	64·1	5·36	5·2	18·2	5·36	5·46		97·552	5·19
0·60	0·75	27	96·1	6·76	6·6	6·4	6·74	3·84		43·136	6·60
0·90	0·75	30	100·0	∞	8·0	0·4	8·30	0·36		3·320	8·00
						35·7		9·66	26·775	184·026	
0	1·04	30	16·8	4·04	4·1	14·1	4·04	0		56·964	4·15
0·30	1·04	24	61·0	5·28	5·5	13·9	5·27	4·17		73·253	5·55
0·60	1·04	31	100·0	∞	7·0	4·1	7·42	2·46		30·422	6·96
0·90	1·04	19	100·0	∞	8·4	0·1	8·67	0·09		0·867	8·36
						32·2		6·72	33·488	161·506	
						103·7		28·89	77·089	527·116	

TABLE 3. *Sums of squares and products for experiment with* T. castaneum

Description	Spray	Film	Total
Swx_1^2	5·190	5·437	10·627
Swx_1x_2	−1·363	−1·326	−2·689
Swx_2^2	5·510	5·821	11·331
Swx_1y	25·390	21·018	46·408
Swx_2y	−0·520	2·043	1·523
Swy^2	135·23	95·57	230·80

lines had suggested that corresponding lines for the two techniques were parallel, and thus that the two probit planes were parallel. The regression coefficients, b_1 and b_2, were therefore derived from the 'Total' column of Table 3, and are the solutions of the equations

$$10{\cdot}627\, b_1 - 2{\cdot}689\, b_2 = 46{\cdot}408,$$
$$-2{\cdot}689\, b_1 + 11{\cdot}331\, b_2 = 1{\cdot}523.$$

For reasons which will shortly appear, it is useful to solve these equations by means of the inverse matrix in the way advocated by Fisher (1941, § 29). It is found that

$$c_{11} = 0{\cdot}10011, \quad c_{12} = 0{\cdot}02376, \quad c_{22} = 0{\cdot}09389,$$

and hence that

$$b_1 = 46{\cdot}408\, c_{11} + 1{\cdot}523\, c_{22}$$
$$= 4{\cdot}682,$$
$$b_2 = 1{\cdot}246.$$

The sum of squares of deviations of y, 230·80, is reduced by an amount

$$b_1\, Swx_1y + b_2\, Swx_2y = 219{\cdot}18,$$

by the fitting of parallel planes to the two sets of twelve points. Values of b_1 and b_2 for the spray and film methods separately are found to be 5·205 and 1·193 for the spray, 4·184 and 1·304 for the film. The portion of Swy^2 accounted for by the fitting of two separate planes is therefore 131·53 for the film and 90·60 for the spray method, a total of 222·13. The difference between the reductions by parallel planes and by non-parallel planes is a measure of the departure from parallelism, and the residual sum of squares is a measure of the heterogeneity of the points about the planes. The total Swy^2 may thus be analysed as follows:

	Degrees of freedom	Sum of squares	Mean square
Regression plane	2	219·18	
Parallelism of planes	2	2·95	
Heterogeneity	18	8·67	0·48
Total	22	230·80	

The total was made up of two sets of eleven degrees of freedom each. The fitting of parallel planes requires two constants and thus removes 2 degrees of freedom, whereas the fitting of two distinct planes removes 4 degrees of freedom in all. The residual, 8·67, may be tested as a χ^2 with 18 degrees of freedom, and is clearly not large enough to suggest any cause of disturbance other than random sampling. Consequently the significance of the departure from parallelism of the two planes may be tested by $\chi^2_{(2)} = 2{\cdot}95$, a value well within the bounds of random variation. Had there been significant evidence for heterogeneity, which nevertheless did not cause the rejection of the hypothesis that the data were representable by planes in favour of some more complex relationship, the test of parallelism would be made by comparing the

ratios of the mean squares for 'parallelism' and 'heterogeneity' in the above analysis by means of a variance ratio test. To allow for such heterogeneity all the standard errors of quantities derived from the analysis would have to be multiplied by the square root of the mean square for heterogeneity, $\chi^2/18$, just as in the simpler case of one dosage factor (Bliss, 1935*a*).

The equations to the probit planes may be written as

$$Y=\bar{y}+b_1(x_1-\bar{x}_1)+b_2(x_2-\bar{x}_2),$$

where $\bar{x}_1=Swx_1/Sx_1$, etc., these being taken separately for the two techniques of spraying and the common values of b_1, b_2 being used. The equations are

$$Y_s=2{\cdot}853+4{\cdot}682\,x_1+1{\cdot}246\,x_2,$$
$$Y_f=2{\cdot}684+4{\cdot}682\,x_1+1{\cdot}246\,x_2.$$

As for the one factor analysis, the variance of the mean probit for each technique is

$$V(\bar{y})=1/Sw;$$

the variances of b_1 and b_2 are, respectively, c_{11} and c_{22}, and the covariance between b_1 and b_2 is c_{12}. From these, the variance of the predicted probit for any dosage is obtainable as

$$V(Y)=1/Sw+c_{11}(x_1-\bar{x}_1)^2+2c_{12}(x_1-\bar{x}_1)(x_2-\bar{x}_2)+c_{22}(x_2-\bar{x}_2)^2,$$

the square root of which gives the standard error of the prediction.

The final column of Table 2 shows the values of Y_s for the dosages used in the experiment. Had these been markedly different from the expected probits used in the fitting, it would have been desirable to repeat the operation of fitting using them as new expected probits and obtaining a second approximation to the maximum likelihood fit; here, the agreement is clearly satisfactory and no further fitting is necessary.

In unifactorial experiments the toxicity of a poison is frequently expressed in terms of the median lethal dose (L.D. 50), the dose for which a 50% kill is predicted by the regression equation. When two dosage factors are involved there is an infinite array of such values obtained by substituting $Y=5$ in the regression equation. Thus for the spray technique the L.D. 50 satisfy

$$4{\cdot}682\,x_1+1{\cdot}246\,x_2=2{\cdot}147.$$

It has been usual to compare the toxicities of two poisons tested over a range of concentrations by means of their *relative potency*, or inverse ratio of concentrations giving the same mortality. When the regression lines of probits on log-concentrations are parallel the relative potency is clearly independent of the mortality, being simply the antilogarithm of the horizontal distance between the two lines. This distance has been denoted by M. So in time or deposit tests the ratio of times of exposure or of deposits giving the same mortality measures a relative potency in a different respect.

When a single experiment involves two or more dosage factors it would be possible to compute relative potencies in respect of each factor separately, the others being held constant. If the two probit regression equations are parallel (i.e. have the same regression coefficients), these relative potencies are all independent of the level of mortality chosen for making the comparison. It is suggested, however, that a more useful comparison between the two poisons is given by the *mean probit difference*, which will be denoted by Δ.

The meaning of this quantity is perhaps a little less readily appreciated than that of the relative potency, but even in the case of a single dosage factor there is some theoretical simplification in using it. Its value is the difference between the predicted probit for the two poisons at a given dosage. When the regression equations are parallel Δ is independent of the particular level of dosage chosen; in fact for a single factor Δ is the vertical distance between the two regression lines, and for two factors Δ is the vertical distance between the two planes.

The mean probit difference may be used to compare the kills produced by the two techniques in the experiment just analysed. If suffixes s and f are used for the two techniques,

$$\Delta=\bar{y}_s-\bar{y}_f-b_1(\bar{x}_{1s}-\bar{x}_{1f})-b_2(\bar{x}_{2s}-\bar{x}_{2f})$$
$$=2{\cdot}853-2{\cdot}684$$
$$=0{\cdot}169\pm0{\cdot}138,$$

the variance of this quantity being obtained as

$$V(\Delta)=1/Sw_s+1/Sw_f+c_{11}(\bar{x}_{1s}-\bar{x}_{1f})^2+2c_{12}(\bar{x}_{1s}-\bar{x}_{1f})(\bar{x}_{2s}-\bar{x}_{2f})+c_{22}(\bar{x}_{2s}-\bar{x}_{2f})^2$$
$$=0{\cdot}0191.$$

For any given dosage the spray technique may be expected to produce a higher kill than the film, though the difference is small. In terms of percentage kill a constant Δ has its greatest value in the middle of the scale, as is shown in Table 4.

TABLE 4. *Comparison of predicted percentage kills by spray and by film techniques with* $\Delta=0{\cdot}169$

% kill by spray	25·0	50·0	75·0	90·0
% kill by film	19·9	43·3	69·3	86·7
% kill by spray	95·0	99·0	99·90	
% kill by film	93·0	98·5	99·83	

It might have been expected that the kill produced by a given dosage would be dependent only on the technique of application and the total amount of pyrethrin deposited on the disk, the latter being obtainable as the product of the total deposit and the concentration of pyrethrin in that deposit. If this were the case b_1 and b_2 would have equal values, since an increase of concentration would have the

same effect as an increase of total deposit in the same proportion. Now

$$V(b_1 - b_2) = c_{11} - 2c_{12} + c_{22}$$
$$= 0{\cdot}146,$$

and therefore

$$b_1 - b_2 = 3{\cdot}436 \pm 0{\cdot}383.$$

There is thus unmistakable evidence that, under the conditions of this experiment, concentration is a far more important factor than deposit in determining the kill. In fact a doubling of concentration was here as effective as an increase in log-deposit by $b_1 \log 2/b_1$, which corresponds to a multiplication of the deposit by 13·5.*

In the two dimensional representation of the fitted planes which is shown in Fig. 1 separate horizontal scales have been used for the two techniques so as to prevent confusion between the two portions of the figure. The steeper set of parallel lines is the regression on x_1 for constant x_2, and the other set the regression on x_2 for constant x_1. From inspection of the positions of the points plotted for the empirical probits relative to these lines it is evident that there is no systematic departure from the linear relationship. This has been found to remain true in further experiments of the same type which have since been performed, and there has been no indication of an interaction between the effect of changes in log-concentration and in log-deposit such as is discussed in § 5.

3. The toxicity of ethylene oxide to *Calandra granaria*

As an example of his method for fitting the probit plane, Bliss (1940) considered data obtained by Busvine (1938) in an experiment on the toxicity of ethylene oxide to *C. granaria*. He obtained the equation

$$Y = -9{\cdot}18 + 11{\cdot}38\, x_1 + 8{\cdot}93\, x_2,$$

where x_1 is the log-concentration of ethylene oxide in mg./l. and x_2 is the log-time of exposure in hours. Using the same series of expected probits, and accepting as correct Bliss's computations on them, a first approximation to the probit plane by the methods of the present paper was found to be

$$Y = -8{\cdot}95 + 11{\cdot}20\, x_1 + 8{\cdot}79\, x_2.$$

The two equations are very similar, as will frequently be the case with data tolerably well fitted by a plane. There are indications of considerable heterogeneity between the batches of insects, since

$$\chi^2_{[35]} = 137{\cdot}0,$$

but little sign of systematic departure from the probits predicted from the plane. These predicted probits were sufficiently close to the first series of expected values used by Bliss to make further computations unnecessary. The standard errors of the regression coefficients, b_1 and b_2, for the plane fitted by the present methods, are $\pm 1{\cdot}13$ and $\pm 0{\cdot}95$ respectively.

4. The toxicity of carbon dioxide to *Tribolium confusum*

As a second example Bliss (1940) used the results of an experiment by Jones (1935), in which batches of 50 or 100 adult *T. confusum* were exposed to atmospheres of 50, 75 and 100% carbon dioxide at 30° C. for times ranging from 3 to 44 hr. The

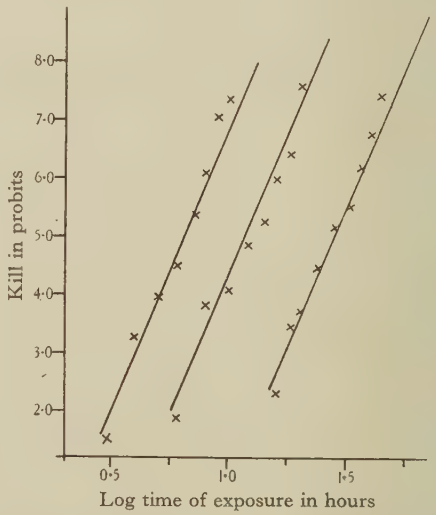

Fig. 2. Toxicity of CO_2 to *T. confusum*. Probit lines obtained by fitting a plane with reference to log-time and log-concentration.

periods of exposure were not the same for the three concentrations and consequently it is not possible to obtain provisional probits by the device used in § 2. If, however, the empirical probits of the percentage kills at each concentration are plotted against log-time (x_2) as in Fig. 2, it is possible to obtain provisional probits from parallel lines drawn by eye through these.

Taking x_1 as the log-concentration of CO_2 in g./l. and following the method of computation of § 2 the regression plane is found as

$$Y = -7{\cdot}99 + 20{\cdot}56\, x_1 + 9{\cdot}69\, x_2,$$

which is very similar to the equation obtained by Bliss

$$Y = -8{\cdot}11 + 20{\cdot}71\, x_1 + 9{\cdot}82\, x_2.$$

* It should not be thought that this is a general truth. In a later experiment, in which the disk on which the insects were placed was covered by a hardened filter paper instead of by an imitation tricoline fabric as here, the effect was completely reversed. The absorptive powers of the substratum almost certainly play an important part in determining the relative effects of changes in concentration and in deposit.

The residual χ^2 measuring the heterogeneity of the observations about the plane is large:

$$\chi^2_{[22]} = 48{\cdot}27.$$

When the three lines given by the plane for the three concentrations used are added to Fig. 2 it is seen that this heterogeneity, in contrast to that of the previous example, indicates systematic departures from the plane, the probits observed for the 75% concentration being generally less than the values predicted by the plane.

This curvature of the regression on x_1 would in general be allowed for by introducing x_1^2 as an additional variate in the analysis and forming the regression on x_1, x_2 and x_1^2. Since only three values of x_1 occur in the experiment, the result of this type of fitting is the same as the fitting of three parallel lines to the three sets of points in Fig. 2 without constraint on their distance apart. If this is done, the residual χ^2 is

$$\chi^2_{[21]} = 32{\cdot}813;$$

a clear improvement has been effected, but even this value is large enough to suggest some disturbing cause. Further investigation shows the assumption of parallelism to be false, the slope for the 75% concentration being less than for the others. The data are satisfactorily fitted by three lines of different slope giving the relationship of probit to log-time for the three concentrations. First approximations to these are:

$$\begin{aligned} 50\%\ CO_2&: Y = -9{\cdot}12 + 9{\cdot}84\, x_2, \\ 75\%\ CO_2&: Y = -3{\cdot}68 + 7{\cdot}93\, x_2, \\ 100\%\ CO_2&: Y = -3{\cdot}70 + 10{\cdot}83\, x_2. \end{aligned}$$

The anomalous behaviour of the second concentration, in respect of both the position and slope of its regression line, suggest either that the effect of concentration on the time relationship is much more complex than that suggested by Bliss, and requires a more extensive experiment for its elucidation, or that experimental conditions during the trials at this concentration differed in some unknown way from those for the other two. The effect, however, is not large.

5. The toxicity of hydrocyanic acid to *Calandra granaria*

The equation $Y = a + b_1x_1 + b_2x_2$

and its obvious extension to three or more dosage factors are the simplest forms of probit mortality-dosage relationships such that the regression of probit mortality on every component of log-dosage separately is linear. When such an equation holds, the effects of the dosage factors are independent and additive. There is, however, a more general equation which still reduces to a linear form when all but one of the dosage measures are held constant. For two factors this equation is

$$Y = a + b_1x_1 + b_2x_2 + b_{12}\, x_1x_2;$$

if x_2 is fixed, Y is linearly dependent on x_1, but the slope of the regression of Y on x_1 increases with increasing x_2 (decreases if b_{12} is negative). The coefficient b_{12} represents the *interaction* of the two factors, since for a unit increase in x_1 and x_2 it shows the increase in Y over and above that which would be expected if a purely additive law obtained. This probit surface may be described in geometrical terms as a hyperbolic paraboloid; sections by planes $x_1 =$ constant or $x_2 =$ constant are generators (straight lines) and sections by $Y =$ constant are rectangular hyperbolas. When more than two factors are involved, it may be further elaborated so as to include terms representing more complex interactions, without destroying its linearity in the individual $x_1, x_2, \ldots$.

Peters & Ganter (1935) tested the toxicity of hydrocyanic acid to adult *C. granaria* at seven different concentrations and several exposure times at each concentration, all tests being made at 17° C. Their data suggest that the slope of the regression of probit mortality on log-time in hours (x_2) decreases with increasing log-concentration in mg./l. (x_1). In examining the results, Bliss (1940) fitted an equation of the form

$$Y = (a + bx_1 + x_2)/(a' + b'x_1).$$

This is linear in x_2 but not in x_1 and therefore differs fundamentally from the usual findings in single factor experiments.

In order to fit a surface of the form suggested at the beginning of this section, it is only necessary first to compute all the products x_1x_2 for the dosages used, and then to treat this product as a third factor, x_3. The computations will not be shown here, as they require only a simple extension of the methods used in the example of § 2. The data as given by Bliss (1940) were used and his column headed 'second expected probit' used as the first set of expected probits. The solution first obtained was used to give a new set of expected probits and the computations repeated with these. Two cycles of the computations gave sufficient convergence of the values of the coefficients, the data for $x_1 = 0{\cdot}778$ which Bliss omitted being introduced in the second cycle; in order to have a more accurate solution, a third cycle was also computed and gave the equation as

$$Y = -9{\cdot}13 + 8{\cdot}31\, x_1 + 8{\cdot}86\, x_2 - 1{\cdot}90\, x_1x_2,$$

and

$$\chi^2_{[23]} = 51{\cdot}27,$$

which shows clear evidence of heterogeneity. The quantities c_{ij} may be set out in the form of a matrix, C, in which for example, c_{23} is the second entry in the third column

$$C = \begin{pmatrix} 2{\cdot}3235 & 2{\cdot}4936 & -1{\cdot}3052 \\ — & 2{\cdot}7818 & -1{\cdot}5106 \\ — & — & 1{\cdot}0889 \end{pmatrix}.$$

Making allowances for the heterogeneity, the standard errors of the three regression coefficients are found to be $\pm 2{\cdot}28$, $\pm 2{\cdot}49$ and $\pm 1{\cdot}56$ respectively.

The coefficient of x_1x_2 is not greatly in excess of its standard error; this fact suggested that the data would be almost equally well represented by a plane, as in the examples previously discussed. If the values of the sums of squares and products already computed are used to fit such a plane, the equation

$$Y = -6{\cdot}03 + 6{\cdot}04\, x_1 + 6{\cdot}22\, x_2$$

is obtained, with $\chi^2_{[24]} = 54{\cdot}48$

as a residual. The difference between the two residuals is

$$\chi^2_{[1]} = 3{\cdot}21,$$

indicating that the inclusion of the term in x_1x_2 does not significantly improve the fit. Indeed the improvement is even less significant than at first appears, since, on account of the heterogeneity of the residuals from the more complex equation, the $\chi^2_{[1]}$ should rather be compared with the mean square residual, 2·23, to give a variance ratio of 1·44 with (1, 23) degrees of freedom instead of being tested as a χ^2.

Using the above equation to the plane to give a new series of expected probits, a second approximation to the plane was computed as

$$Y = -6{\cdot}15 + 6{\cdot}10\, x_1 + 6{\cdot}29\, x_2,$$

with $\chi^2_{[24]} = 53{\cdot}10.$

This equation clearly fits the data as well as the more complex form allowing for interactions, as may be further seen by computing from each the expected probits for each level of dosage. Elimination of the unnecessary term in x_1x_2 increases the accuracy of estimation of the remaining coefficients, the standard errors of b_1 and b_2 being $\pm 1{\cdot}30$ and $\pm 1{\cdot}24$ respectively.

It appears, therefore, that there is no evidence of interaction between the log-concentration of hydrocyanic acid and the log-time of exposure in their effects on mortality and hence no necessity to invoke the more complex equation in preference to the plane. There was undoubtedly heterogeneity between batches of insects in their reaction to the poison. This heterogeneity was noted by Bliss in his examination of the data, but he did not test whether or not a simpler hypothesis could represent the data as satisfactorily as his equation.

6. Discussion

The method of probit analysis is a particular form of regression analysis with the additional complication that the weights to be attached to the observations are dependent on the regression equation. Consequently the regression technique for fitting multivariate or curvilinear regression equations can be adopted for use with probits. In § 2 the method for deriving the linear regression of probits on two independent variates representing two separate dosage factors has been fully described. This method, while no more tedious to compute than that proposed by Bliss (1940), has the advantage of being precisely analogous to the univariate case and being easily generalized to the analysis of data containing three or more independent factors. If the various sums of squares and sums of products of deviations are systematically calculated, no difficulty should be experienced in making the generalization for any number of factors. The test for parallelism of probit planes and the representation of a probit plane by two sets of parallel lines are likewise capable of immediate extension, as also is the concept of mean probit difference as a measure of difference between the potencies of two methods or materials, instead of the usual measure by means of relative potency.

When the probits of the kills are not linearly related to the dosages (whether one or more factors are involved) the nature of the curvilinear relationship which exists may be investigated by introducing squares and higher powers of the measures of dosage, and their products, and treating these as separate factors in the analysis, for which appropriate regression coefficients have to be obtained. It is possible that situations may occur in which probits are linearly related to each dosage factor separately, but the form of that linear relationship changes with changing levels of the other factors. A more general equation has been proposed to take account of such interactions between factors when only two are involved. The extension of this to the case of three factors gives the equation

$$Y = a + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{123}x_1x_2x_3,$$

in which the regression coefficient b_{12} measures the interaction between the first two factors and b_{123} measures the three factor interaction. The method of fitting such an equation is to treat the four products as though they were separate factors. This again may easily be extended.

I am greatly indebted to Dr F. Tattersfield and Dr C. Potter, of the Insecticides Department, Rothamsted Experimental Station, for permission to make use of their unpublished data on the effect of concentration, deposit, and method of application of a pyrethrin oil spray on the mortality of *Tribolium castaneum*.

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(*Received* 17 *June* 1942)

Rhizoctonia solani associated with a root rot of cereals in Norfolk

By W. A. R. DILLON WESTON, *School of Agriculture, Cambridge*
AND S. D. GARRETT, *Rothamsted Experimental Station, Harpenden*

In 1938, our attention was drawn to partial failures of cereal crops in Norfolk; such failures were most frequently reported in barley, but occurred also in wheat and in oats. Patches of backward and stunted plants became noticeable in affected crops soon after growth had started or been resumed in the spring. The affected areas were usually small and sharply defined, but occasionally covered $\frac{1}{4}$ acre or more. Some or all of the plants in these patches were usually dead or dying by mid-June, but others which survived were much delayed in growth and easily distinguishable from the rest of the crop at harvest by their green colour.

The roots of diseased plants were infected by *Rhizoctonia solani*, and the condition appeared to be similar to that described by Samuel & Garrett (1932) from South Australia, and by Hynes (1937) from New South Wales. This diagnosis was confirmed by isolation of *R. solani* from affected plants, and by pathogenicity tests with the fungus. Like the Australian authors, we have never observed the fungus to infect the stem, only the roots being attacked; the killing short of the first-formed crown roots is the most obvious effect upon the root system.

Hynes (1937) drew attention to the way in which Simmonds & Sallans (1930, 1933) reproduced above-ground symptoms of different types of cereal root rots by their root-cutting studies. Simmonds & Sallans concluded: 'The reactions expressed by plants receiving seminal root or sub-crown internode amputations are comparable with symptoms of a root-rot disease which causes poor tillerage and late maturity. Crown root amputation reactions, on the other hand, are comparable with root-rot diseases which produce early maturity.' Now the *R. solani* disease, which destroys the seminal roots and the early-formed crown roots, is responsible for late maturity of recovered plants; in the same category can be placed the browning root-rot disease of wheat in Canada, due to *Pythium* spp. (Vanterpool, 1940). On the other hand, the take-all disease of wheat, due to *Ophiobolus graminis*, does not usually reduce very much the efficiency of the seminal and crown-root systems during the first part of the growing season, but produces maximum root damage at heading time; as with the crown-root amputations of Simmonds & Sallans, this disease produces early maturity.

The *R. solani* disease of cereals is only of minor economic importance in this country; it has been recorded only from seven farms in Norfolk. Nevertheless, its occurrence is of some general interest in view of its apparent identity with the Australian disease, and we are investigating the condition further to determine possible predisposing factors.

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(*Received* 29 *July* 1942)

Proceedings of the Association of Applied Biologists

A Joint Meeting of the Association of Applied Biologists and of the British Mycological Society was held on Friday, 9 Oct. 1942 in the London School of Hygiene and Tropical Medicine. The morning session commenced at 11 a.m., the Chair being taken by Miss E. M. Blackwell, M.Sc., President of the British Mycological Society. The afternoon session commenced at 2 p.m., the Chair being taken by Dr Hubert Martin, President of the Association of Applied Biologists.

Discussion on potato virus diseases

Morning session

I. Potato virus diseases: introduction. By G. SAMUEL.
II. Some properties of the potato viruses. By F. C. BAWDEN.
III. Some practical difficulties in the production of virus-free seed potatoes. By KENNETH M. SMITH.
IV. Some factors influencing the health of seed potato stocks in North Wales. By T. WHITEHEAD.

Afternoon session

V. Ecology of potato aphides in North Wales. By I. THOMAS and F. H. JACOB.
VI. The life history of *Aphis* (Doralis) *rhamni* in Eastern England. By J. P. DONCASTER.
VII. The spread of potato viruses in the field. By P. H. GREGORY.
VIII. Potato breeding for virus resistance. By GEORGE COCKERHAM.

I. Potato virus diseases: introduction

BY G. SAMUEL, M.Sc.
Ministry of Agriculture and Fisheries, Plant Pathological Laboratory, Harpenden

(With 1 Text-figure)

The purpose of this introductory paper is to sketch in some of the background, more especially on the practical side, so that those not so familiar with the subject may derive the greatest interest from the research papers which follow.

It is suggested that potato virus diseases are by far the most interesting problem in plant pathology in this country. With present acreages, each year more than 300,000 tons of Scottish and Irish seed potatoes are imported into England, and within the space of 2 or 3 years nearly the whole of this seed has been discarded as unsuitable for further growth. No other plant disease in the country is responsible for such a tremendous destruction of plant material. One cannot help wondering what we should do if Scotland, Ireland and Wales were to float away and leave England all to itself. We should then be forced to 'turn to' and control our own virus diseases—and possibly the process might be very good for us. At all events, the present system means that under existing conditions the import of fresh seed from Scotland and Ireland is the most economic, or at least the easiest, method of controlling the virus diseases. Fig. 1 gives an idea of the extent to which this import of fresh seed is practised in different parts of England. It is evident that in the counties coloured black a large number of farmers find it worth while to purchase new seed from Scotland or Ireland every year; in counties cross-hatched the seed may be said to last on the average for 2 years; in those with horizontal lines about 3 years; in those with broken lines about 5 or 6 years; and in those counties in Wales that are white some 10 or more years—a fact which suggests that they might be self-supporting in seed if they wished, and probably supply the whole of Wales.

The big trade involved in producing and distributing seed potatoes from Scotland and Ireland has been much helped by the official schemes of health certification instituted in those countries. Certification for freedom from virus diseases began in Scotland only in 1931, when the standard required was that no more than 5% of the plants should be affected with leaf-roll or severe mosaic. The standard was soon raised to 3%, and in 1937 a higher grade certificate was instituted with a standard of no more than 0·5% of leaf-roll and severe mosaic, with no more than 1% of all easily visible virus diseases. The institution of this higher grade certificate has stimulated roguing of crops on a practical scale (many farmers now hire professional roguers), and in the absence of appreciable insect transmission this re-

sults in a definite improvement of stocks. Over 20,000 acres each year now reach this higher grade. The highest grade Stock Seed certificate began as a special report for stocks built up from selected plants; and although this feature has not been continued the standard required is still very high—viz. no more than 0·25 % of visible virus disease, with less than four plants per acre—about 0·025 %—of leaf-roll or severe mosaic. Some of this highest grade seed is bought by ware growers in England and planted in counties where it degenerates in one year. On general grounds this would seem an inexcusable waste of foundation seed, which would be better kept in Scotland to displace the poorer stocks that are there (see data in Cockerham, 1939).

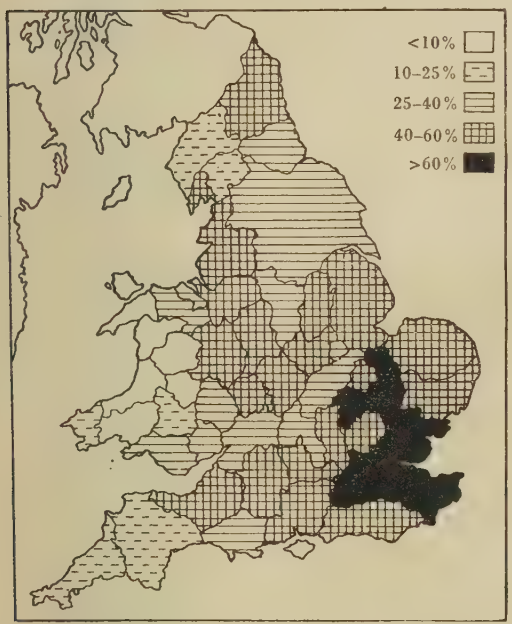

Fig. 1. Proportion of Scotch and Irish seed potatoes planted in counties in England and Wales, 1942.

Although the position in respect of the insect-transmitted viruses leaf-roll and severe mosaic (virus *Y*) in Scottish and Irish seed is now very good on the whole, it is less so in respect of mild mosaic (virus *X*) which is present throughout most stocks of certain varieties in a form so mild that it is often difficult or impossible to recognize with the naked eye. Nevertheless, Scott (1941) has shown that it may, at least in certain circumstances, cause a reduction in yield of from 16 to 40 %. The main problem in the production of the highest quality seed that now remains to be solved is thus to multiply stocks from tested *X*-free tubers on a large commercial scale. That it can be done on a relatively small scale was shown by Clinch *et al.* (1938), who found that stocks of Champion potatoes multiplied from tested tubers sent to Donegal were still free from virus *X* in seven commercial fields 4–6 years later. Whether it will be possible to multiply tested virus-free stocks of other varieties to the stage of thousands of acres without their becoming contaminated with the insidious viruses of the *X*-group, remains to be proved. Unfortunately, affected plants cannot be rogued out because they cannot usually be seen. An attempt is now being made to multiply in this way the virus-free stocks produced by Dr Salaman in the Cambridge glasshouses. The difficulty will be not so much to keep them free from the insect-transmitted viruses as from virus *X*, the means of dissemination of which in the field cannot be said to be fully understood even now, though Loughnane & Murphy (1938) showed it could occur by leaf contact.

So much for the seed problem. The other part of the potato virus problem, and perhaps the more difficult, is to make good seed last as long as possible when brought to England for ware-growing. The rapid degeneration that sets in in south-eastern England is due almost entirely to the spread within the stocks of the two insect-transmitted viruses leaf-roll and virus *Y*. The two main aphis vectors concerned are *Myzus persicae* and *Aphis rhamni*. Winged forms of these aphides introduce the diseases even into the best of stocks, and the crawling, apterous forms spread them fairly rapidly from infected plants to a number of surrounding healthy plants (though there are certain differences in this respect with which Mr Bawden will deal). The problem is immensely difficult because so many factors are concerned, e.g. rate of multiplication in the crop of the insect vectors, which depends largely on the earliness of invasion and thus on the proximity of overwintering sources (peach trees, greenhouses or sometimes Brassica crops for *Myzus persicae*, and *Rhamnus* bushes for *Aphis rhamni*); proximity of other potato crops containing virus diseases; infected ground-keepers among the potatoes, where the rotation is too close, or possibly also in neighbouring corn crops; possibility of there being reservoirs of virus in weed hosts; varietal differences in susceptibility, etc., etc. Although we have general knowledge on a number of these points it is not as yet nearly specific enough, even for the main ware potato-growing district of Lincolnshire. It is to find out the facts, on which any scheme of improvement must eventually be based, that Dr Gregory and Mr Doncaster have been appointed by the Agricultural Research Council to investigate the virus and the insect sides respectively, and some of the first results of their work will be described in later papers.

Meanwhile, the most that the Ministry of Agriculture and Fisheries has felt able to do in regard to English-grown seed, apart from encouraging the development of seed potato growers' associations in suitable areas in Wales and the north and west of

England, is to institute a scheme of certification of 'once-grown' seed. The essential features of the scheme are that the parent stock shall be a good Scotch, Irish, or English or Welsh seed growers' association stock, containing less than 3% of leaf-roll and severe mosaic, which is also isolated more than 50 yd. from other potatoes containing these diseases. This scheme is encouraging the purchase of the better quality stocks from Scotland and Ireland, and also influencing commercial practice towards growing such good stocks in isolation. Formerly, it was an almost universal practice to plant new seed in the same field as once- or twice-grown seed, often containing an appreciable proportion of virus-affected plants, but there is evidence that the better growers are now abandoning this practice. That certified once-grown stocks may in general be expected to give good yields is shown in Table 1, where the yields of a few English-grown certified King Edward stocks chosen at random are compared with that of a Scottish stock bearing a high grade certificate.

As the knowledge obtained by the research workers increases it should become possible to do considerably more towards promoting the most economical use of the improved seed that is now becoming available.

TABLE 1. *English certified (once-grown) stocks of potatoes from various localities, grown at the Midland Agricultural College, 1941. Disease and yield records*

Variety: King Edward

Locality grown in 1940	% severe virus	Yield (tons/acre)	Yield as percentage of control	
Northampton	1·5	18·9	119	
Cornhill-on-Tweed	0·0	17·2	109	
Cople, Beds	3·0	16·8	106	
Haxey, Lincs	5·0	16·4	103	
Holbeach, Lincs	1·0	16·3	102	
Dumfries, Scotland	*0·0*	*15·8*	*100*	*Control*
Croxton, Cambs	1·0	15·8	100	
Gresham, Norfolk	10·0	14·9	94	
Camston, Notts	3·0	14·5	92	
Nettleham Heath, Lincs	14·5	14·1	89	
Eye, Peterborough	4·0	10·7	67	

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II. Some properties of the potato viruses

BY F. C. BAWDEN, M.A.

Rothamsted Experimental Station, Harpenden, Herts

Of the twenty potato viruses that have been described, for practical purposes only three need consideration: these are leaf-roll, *X* and *Y*. The others either do not occur in the British Isles or else they occur either rarely or cause little loss. Some of these relatively unimportant viruses are also undoubtedly strains of *X* and *Y*. For example, viruses *B* and *D* are serologically related to virus *X*, and *C* to *Y*, although they may cause strikingly different diseases in the same varieties and be transmitted differently.

Economically, leaf-roll is the most important virus, for although it may be less effective than virus *Y* in reducing the yield of individual plants, it occurs much more frequently. It produces the same type of symptom in all commercial varieties: typical infected plants are dwarfed, upright and chlorotic, while the leaflets are harsh and roll upwards and inwards to expose under-surfaces that are often pigmented. There is, however, considerable variation in the severity of symptoms shown by individual plants of the same variety. The reasons for this are unknown. The most likely is that leaf-roll, like most other viruses, exists in strains of varying virulence, but there is no proof of this yet, and the variation in the symptoms of secondary leaf-roll plants may arise from other causes. Possibilities are: variations in the length of time the plant has been infected, in size of sett or in nutrition. Typical plants are easily diagnosed, but plants with only slight pallor or rolling, or those having an unnaturally upright habit, can easily cause confusion in field inspections, especially as these symptoms often arise from causes other than infection with leaf-roll virus. In 1913 Quanjer described phloem necrosis as a symptom of leaf-roll, and recently Miss Sheffield has found this to be a sufficiently constant and specific feature to be of diagnostic value.

In most varieties virus *Y* causes leaf-drop streak in the first year of infection and a severe mosaic in second and subsequent years. In some varieties,

however, e.g. Arran Pilot and Arran Victory, it causes only a mosaic in the first year of infection. In varieties such as Majestic, which show leaf-drop streak in the current year of infection, there are considerable differences in the severity and type of symptoms shown by individual plants grown from infected tubers. Some plants are so crinkled and dwarfed that they approximate to the condition known as curly dwarf. Others are less crippled, but they show definite mottling and soon become prostrate and sprawl untidily. In some there is often necrosis and shrivelling of the lower leaves, and all mature much earlier than healthy plants, so that in late field inspections they are apt to be missed.

The symptoms caused by virus *X* vary widely with different potato varieties and with different strains of the virus. One strain may kill one variety and cause only a mottle in another, while a second strain may reverse the procedure. In the field, virus *X* is found commonly only in varieties in which it causes interveinal mottling, but in these it is usually widespread. The symptoms may be insufficient to be noticed readily, especially if the whole crop is uniformly affected as many crops are, but such infections have been found to reduce the yield by 20%.

These three main virus types differ from one another in their other properties as much as in the symptoms they cause. A knowledge of these properties, and especially of the methods of transmission, is necessary before their behaviour in the field can be interpreted and control measures devised. Viruses *X* and *Y* are both anisotropic nucleoproteins and are readily transmitted mechanically. Virus *X* is more resistant to heating and ageing and occurs in the sap of infected plants at a higher concentration. Virus *Y* is transmitted by the aphides *Myzus persicae*, *Aphis rhamni* and *Macrosiphum gei*, but none of these transmits virus *X*, for which no vector is known. In this connexion it may be worth noting that virus *X* is hydrolysed by proteolytic enzymes that do not destroy virus *Y*. Nothing is known of the nature of leaf-roll virus or of its properties in vitro, for it is not transmitted mechanically. It is transmitted by the aphis *Myzus persicae*.

In the field it is common for one of the aphis-transmitted viruses to spread considerably while the other does not, or for one to spread at one time of the season and the other at another time. When virus *Y* spreads and leaf-roll does not, a possible interpretation is that aphides were present which could act as vectors of *Y* but not of leaf-roll. Often, however, leaf-roll spreads while *Y* does not, and then no such simple explanation is forthcoming. A knowledge of the relationships of the two viruses with their vectors supplies a possible explanation. Aphides become infective with leaf-roll only slowly. They have to feed for a considerable time on the infected plants, and then a day or so must elapse before they can infect a healthy one. When the vectors have become infective they remain so for relatively long periods, often for the remainder of their lives. Mrs Watson has shown that the relationships of virus *Y* with its vectors are very different. Aphides can transmit this virus immediately after feeding on an infected plant, and their efficiency as vectors is greatly decreased by continuous feeding either on healthy plants or infected plants. The optimum conditions for transmission are obtained by using aphides that have been starved for some time and then fed for only a few minutes on the diseased plant. Vectors of virus *Y* remain infective for much shorter periods than vectors of leaf-roll, but the exact duration of infectivity varies with the feeding of the vectors. Those which feed continuously, either before or after access to an infected plant, lose their infectivity in a few minutes, whereas those which do not feed continuously may remain infective for some hours. These facts suggest that the winged migrants, which will be fasting when in flight and alight to feed for only short periods, are those most likely to fulfil the requirements for optimal transmission of virus *Y*, whereas the slow-moving apterous aphides, which spend long periods on individual plants, are most suitable for spreading leaf-roll.

It is these two aphis-transmitted viruses that are responsible for the rapid degeneration of potato stocks in the south and east of England, and seed from Scotland and Ireland is superior because of its relative freedom from them. Except for varieties which die when infected with the more common strains of virus *X*, however, such seed is usually widely infected with virus *X*, for this seems to spread as readily in Scotland and Ireland as in England. How this spread occurs is still not satisfactorily explained. In Ireland it has been shown that healthy plants can become infected if their leaves are rubbed against infected plants. This method of transmission would possibly soon lead to a whole stock becoming infected, especially in the wind-swept districts which are now used for seed growing because of the absence of aphides, but there is some doubt if it can account for all infections. Virus *X* has frequently been found in crops of *Hyoscyamus* and tomatoes, both under glass and in the open, and there would seem little chance for these to have rubbed against infected potatoes. In the present state of our knowledge about the methods whereby virus *X* is spread in the field, it is impossible to define conditions in which spread will not occur. Thus, although the propagation of stocks free from this virus is of considerable economic importance, further research will be needed on the methods of transmission before any such schemes can confidently be started on a commercial scale.

III. Some practical difficulties in the production of virus-free seed potatoes

By Kenneth M. Smith, D.Sc., F.R.S.
Plant Virus Research Station, Cambridge

The Potato—now the Plant—Virus Research Station was started in Cambridge in 1926. Its work was to consist of two parts, one to carry on research upon viruses, especially those affecting the potato plant, and the other was to build up and maintain a stock of virus-free potatoes. This latter part of the work was in charge of Dr R. N. Salaman who built up the foundation stocks and kept them going until his retirement in 1939. The work was then taken over by my colleagues and myself.

The first of our practical difficulties is that of obtaining really virus-free material of new potato varieties as they appear. It frequently happens that out of a whole batch of a new variety tested, all are already infected with potato virus *X*. Perhaps the secret is to obtain samples of new varieties at the earliest possible moment. There are certain well-known potato varieties which can be obtained in a virus-free condition, only with the greatest difficulty. Up-to-Date, for example, always has virus *X* or Up-to-Date streak and Golden Wonder is almost invariably infected with viruses *X* or *A*. Just lately we seem to have acquired some virus-free Up-to-Date from Australia through the kindness of Dr J. G. Bald.

Another of our troubles is concerned with that elusive virus known as potato virus *X*. This virus quite frequently turns up in potato plants which have been previously carefully tested and which have never been outside the glasshouse. In consequence we have always to be on the look out for the tell-tale mottle which indicates its presence. Although Loughnane & Murphy demonstrated that this virus can pass from diseased to healthy potato plants by contact, it is possible that this is not the only method of spread. Another possible explanation of the sudden appearance of this virus is that it may exist in an extremely non-virulent and masked form which might be missed in inoculation tests and that a more virulent variant might arise from this masked form and so become apparent. Whatever be the true explanation, the fact remains that the *X* virus is a constant source of trouble in the glasshouse. Incidentally, it would be worth carrying out tests to see how far this virus can be transmitted from tuber to tuber by means of a tuber-cutting knife.

It may be worth while to give a few details of how we test for viruses in the potato plant. Various solanaceous plants are used which give a quick and unmistakable response to infection with the more common sap-inoculable viruses. Tobacco, *Nicotiana glutinosa* and *Capsicum*, all react well to such viruses as *X* and *Y*. For other viruses which are not sap-transmissible we use grafting and a variety of potato which shows marked symptoms of the virus being tested. Most of the testing is carried out in the spring, but we have also used the tuber-indexing method in the winter. In this method an eye is forced in sand and the resulting shoot tested in the usual way. We have found this method rather unreliable, probably owing to the fact that some viruses seem to be rather slow in coming up into the shoot, and we have sometimes failed to check up the leaf-roll and *Y* viruses by this method.

There is one more practical point regarding the glasshouse procedure, and that relates to the occasional entrance of insects which will happen, however good the insect-proofing may be. The two chief insect invaders are *Myzus persicae* and *Thrips tabaci*, but these can be kept under control by regular fumigation.

Those, very briefly, are the difficulties which we have experienced in growing virus-free seed potatoes under glass. They are not very serious and can be overcome, but the maintenance of the stocks requires constant vigilance and routine testing. The most important point is to be sure that the original potato stocks are really virus-free in the first place.

The real difficulties commence when we try to multiply up our glasshouse stocks out-of-doors. We have tried several types of isolation, but none has proved very successful or practical. Dr Salaman favoured isolation in the centre of a field of corn. A number of small plots were planted in a field of wheat or barley, the idea being that the tall corn would screen the potato plants from wandering aphides.

Another more ambitious attempt was carried out in the 'fire drives' of the forests on the east coast. Here we could obtain almost complete isolation from other potatoes, and the tall trees on either side of the drive seemed an effective screen. The amount of land available was almost unlimited, and we chose a drive which had had the gyrotiller over it and we manured it heavily. The first difficulty was the soil which was almost pure sand. In spite of manuring and the addition of artificials, deficiency diseases developed which not only obscured any virus diseases which might be present but also simulated them, particularly leaf-roll. This made roguing virtually impossible. As regards aphis infestation we found no *Myzus persicae*, but *Aphis rhamni* and *Myzus pseudosolani* were abundant and they would be capable of spreading any *Y* or leaf-roll viruses if already present in the crop.

Another difficulty was inherent in the extreme

isolation of the plots which meant that the plots lacked supervision, they could not be hoed regularly, and spraying for blight was almost impossible. I should perhaps mention that so far as we were concerned it was necessary for the isolation plots to be within 50 miles or so of Cambridge so that it should be practicable to exercise adequate supervision. This added restriction made the difficulties almost insuperable, and we have for the time being given up the attempt to multiply our virus-free stocks in England. We have now entered into an arrangement with the National Institute of Agricultural Botany by which our glasshouse stocks are grown in isolation in Northern Ireland by competent seed-potato growers and they are subjected to continued scrutiny by expert potato inspectors. Each year therefore we supply nucleus stocks to the National Institute of Agricultural Botany for multiplication in Ireland, and when sufficient stocks have been built up they will be passed on to the seed trade.

IV. Some factors influencing the health of seed potato stocks in North Wales

By T. Whitehead, Ph.D., M.Sc., A.R.C.Sc.
University College of North Wales, Bangor

(With 6 Text-figures)

This communication discusses some of the more important factors which appear to have affected the health of seed-potato stocks growing under a scheme which has been in operation in North Wales for the last 15 years. After a preliminary year's trial with three varieties on eight farms, the scheme was put on a proper basis in 1928 by the allocation of a grant from the Ministry of Agriculture and Fisheries and by the appointment of Mr J. F. Currie as Supervisor. In that year some forty-five stocks (ex Eire) of the same three varieties (Sharpe's Express, Great Scot, and Kerr's Pink) were planted on fifteen farms in Anglesey, Flintshire, Denbighshire, and Caernarvonshire. The scheme has been developed along lines suggested by the acquisition of scientific data, until we now have 268 stocks of some eleven varieties growing on ninety-five farms; the location of those in Anglesey and Caernarvonshire is shown in Fig. 1.

The conditions under which the seed potatoes are produced include the following points which seem of importance in the present survey:

(1) Only authorized stocks may be planted. Apart from initial stocks of new varieties, which are purchased by the College from the healthiest sources on the grower's behalf, the effect of this condition has been to ensure that new centres are planted with stocks from farms already under the scheme, and which have therefore been subject to North Wales conditions since their introduction.

(2) For the first half of the period under review each crop was inspected three times during growth by the Supervisor, or one of the student temporary Inspectors. With the increase in acreage, however, the inspections were reduced to two, the second one being carried out by the Supervisor.

(3) During inspection, every plant is examined and the percentage disease in the crop is obtained from the sum total of visibly affected plants noted throughout the season. All forms of virus diseases are recorded except the extremely mild symptoms usually referred to as 'negligible mottle'.

(4) Every plant recorded as diseased, together with its tubers, is removed by the Inspector at the time it is noted; so that the stocks, as sold, contain only such diseased tubers as result from masked symptoms or current season infection.

(5) During the first half of the period reliance was placed on this personal 'roguing' by the Inspector as a means of maintaining the health of the stocks, and a crop was not discarded unless it was growing in close proximity to a badly diseased stock or was, itself, infected to the extent of upwards of 2%. In accordance with the provisions of the Seeds Amendment Act 1935, however, the additional precaution has been taken since 1936 of prohibiting the planting of seed from any crops exceeding 1% virus infection. The value of this 'planting standard' will become apparent later.

Nature and degree of reliability of data

For the purpose of this analysis fifty-nine nuclear stocks (i.e. initial stocks as introduced from Scotland or Eire) of the eight most widely grown varieties have been followed up, and the percentages of leaf-roll and diseases of the mosaic type noted for each year, both at the original centre and on all farms under the scheme to which the stock was distributed; the whole forming what may conveniently, if inaccurately, be described as a 'pedigree' for that stock. One such 'pedigree' is illustrated in Fig. 2, from which it will be seen that on only fourteen occasions did the combined virus infection exceed 1%, on three of which it reached 3%. This typical example of the high standard of health of the original varieties grown under the scheme is not quite so easily maintained with the more recently introduced kinds, as will be seen later, but, even so, Table 1 indicates that a considerable degree of success has been achieved in the maintenance of the health of all the seed stocks.

Since the conclusions reached in this analysis are based on the inspection figures for disease recorded

Fig. 1. Location of seed-producing farms in Anglesey and Caernarvonshire. M, Aa–d, Ca–d = Topographical zones. D, F, G = Seed-farms at which aphis counts are made. C = University College farm at which aphis counts are made.

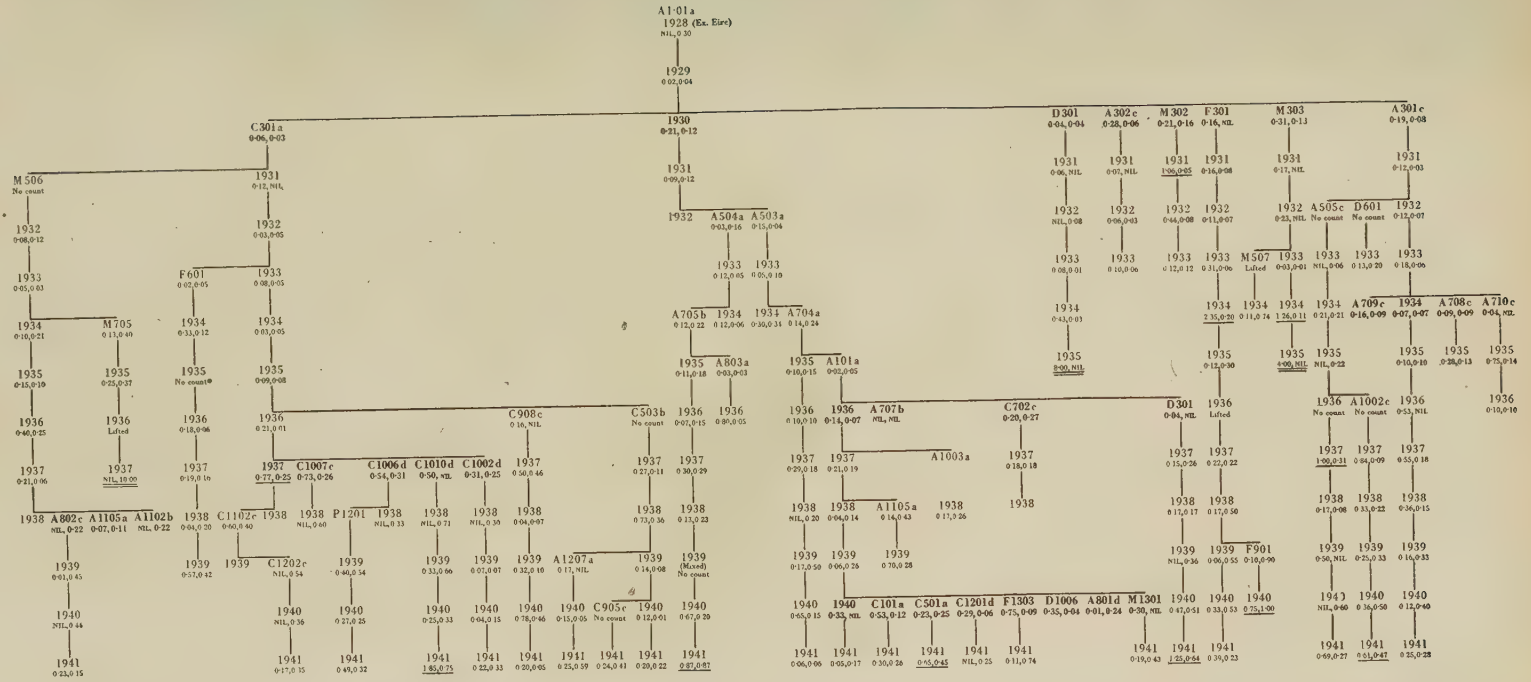

Fig. 2. Distribution and health of one nuclear stock of Sharpe's Express (1928–41). Each farm is designated by a symbol in which the letters indicate its topographical zone. The percentage virus disease is given for each year; leaf-roll on left and 'mosaic' on right. Heavy type = examples of disease on crops of same immediate origin on different farms. Total virus disease exceeding 3% is doubly underlined. Total virus disease between 1 and 3% is underlined. * = Crop rejected in 1935 owing to proximity of diseased stock.

in the fifty-nine 'pedigrees' it is important to consider the degree of reliance to which they are entitled. This is, perhaps, best assessed by the degree of uniformity in the inspection figures for crops of the same origin and growing in their first year on a number of different farms. For this purpose, also, it is advisable to take the leaf-roll figures alone since this eliminates any variation due to the symptoms from different amounts of current season infection with 'mosaic'.

TABLE 1. *Health of stocks under the North Wales scheme*

Compiled from data kindly supplied by Mr J. F. Currie.

Year	% stocks with less than 1 % total virus	% stocks with 1–3 % virus infection	% stocks exceeding 3 % infection
1928	90	10	0
1929	89	11	0
1930	79	10	11
1931	91	7	2
1932	97	2	1
1933	100	0	0
1934	90	8	2
1935	65	24	11
1936	68	23	9
1937	84	13·5	2·5
1938	87	12	1
1939	85·7	12·2	2·1
1940	78	19	3
1941	70	26	4
1942	82·9	13·8	3·3

Apart from the personal error in diagnosis by the Inspector, which we are in fact attempting to measure, there are real causes of variation due to (*a*) sprout infestation in storage by aphides, (*b*) diseased ground-keepers, and (*c*) errors in the estimation of the total number of plants in the crop, on which the percentage virus infection is based. Of these causes of variation (*a*) and (*b*) are readily detected by growing small 'check' plots on the College Farm from samples of tubers from each farm. Neither cause is believed to have been of any real importance although in one case a fifteenfold increase in leaf-roll was attributed to sprout infection. As regards (*c*), the total number of plants is estimated by the Inspector and checked by the Supervisor, who takes the lowest probable figure in order to safeguard the standards of health. Whilst bearing all these points in mind, it is believed from a study of the fifty-nine pedigrees that a reasonable degree of reliance can be placed upon the inspection figures; a typical example is seen in Fig. 2 in which the relevant data are shown in heavy type. Nevertheless, Fig. 2 also shows from the percentages of leaf-roll recorded in the stocks of Sharpe's Express distributed from farm A 101 *a* in 1940, that a variation of more than 100 % either way from the amount of disease found on the distributing farm may occasionally be met with, for whereas the distributing farm showed 0·33 % leaf-roll, 0·75 % was found in the same stock growing on farm F 1303 and only 0·01 % on farm A 801 *d*.

Relationship between aphis counts and crop degeneration

(*a*) *General.* The first relationship to be examined was that between the aphis population during the period 1928–42 and the number of seed potato stocks with a virus infection exceeding 1 %. Fig. 3 gives the 'Index Figures' of *Myzus persicae* (i.e. the number per 100 leaves taken at random in mid-July) at five centres.* All except one lie on a coastal line running from the Dee estuary south-west to the tip of the Lleyn peninsula some 80 miles away. Centre J (Berthymaen) is situated at an altitude of 400 ft. at Trelogan, on the Holywell plateau of Flintshire and overlooking the Dee estuary. Some 12 miles slightly south of west from centre J lies centre I (Ty Mawr) on the sea coast near Rhyl in Denbighshire. The College Farm (centre C) is a further 20 miles westward at Aber, on the sea coast and overlooking the north-eastern entrance to the Menai Straits. Twenty-four miles slightly north of west from Aber lies centre D (Ty Wridin, farm A 101 *a*) on an exposed coastal bluff near Holyhead in Anglesey. Centre G (Hendre, farm C 101 *a*) is at the tip of the Lleyn peninsula near Aberdaron, some 30 miles across Caernarvon Bay from centre D and 42 miles south-west from Aber. Counts were also made in many years at centre F (Madryn, farm C 102 *b*) 9 miles north-east from Aberdaron and situated at an altitude of 300 ft., some 4 miles from the coast and in thickly wooded country. The aphis counts at centre F approximate to those at centre G and are not shown in Fig. 3; the location of all except the first two is given in Fig. 1.

Taking North Wales as a whole, Fig. 3 shows that aphis peaks in population occurred on three occasions during the 15 years, in 1929, 1933 and 1939. Three similar peaks in crop degeneration (in 1930, 1935 and 1940), are also evident in the lower part of Fig. 3, in which the number of stocks of the four varieties continuously under the scheme and exceeding 1 % virus infection, is plotted.† There seems no doubt from this evidence that a definite correlation exists between the population of *M. persicae* and the degree of degeneration in the crops.

(*b*) *Geographical variation in aphis numbers and in degeneration.* Fig. 3 shows that the index figures for *M. persicae* vary widely, not only year by year in the same district, but from one locality to another in the same year. This provides an opportunity for a closer

* The 'index figures' from 1938 onwards have been kindly supplied by Dr I. Thomas; the earlier ones are mostly from the late W. M. Davies's field book.

† The infection graph is based on number, rather than on percentage, of stocks to avoid giving undue prominence to the effect of the 1929 aphis peak when the total number of stocks was very small.

examination of the relationship between aphis numbers and degeneration by cross references between Figs. 3 and 4. In the latter, the percentage of the total number of crops of eight varieties under the scheme, showing more than 1 % virus infection, is allocated to the different localities in which the degeneration is manifest (see also Fig. 1). Thus by adding together the column heights for 1930 we find that, of the small number of stocks then growing, 29 % showed 1 % or more of infection. Of these stocks, 12 % with more than 3 % infection occurred as compared with the steadily increasing number in Anglesey and south Caernarvon. Similarly, the very high standard of health maintained throughout the whole 15 years in Anglesey is certainly related to the absence of evidence of anything deserving the name of 'aphis peak' in that county, even in 1939 when, although exact figures are not available, it is known that aphis numbers were very low. There were no farms under the scheme in the Menai Straits area (zone M) until 1931 but, following on the definite, though relatively low, aphis peak at the College Farm

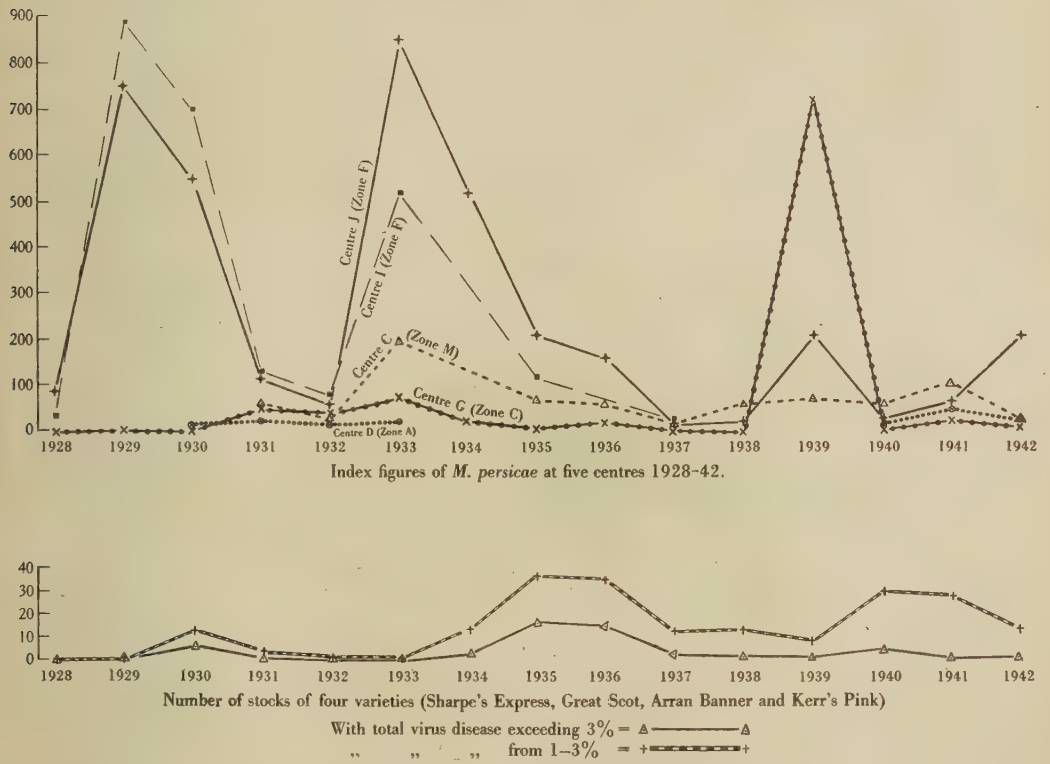

Fig. 3. Relationship between aphis numbers and crop degeneration.

in Flintshire, 2·5 % of equally affected stocks was to be found in central Anglesey (zone Ad of Figs. 1 and 4), whilst the remaining 14·5 %, showing between 1–3 %, was confined to Denbighshire.

Considered as geographical units one would expect degeneration in Flintshire and Denbighshire to show a correlation with the aphis index figures at centres J and I, and in fact the stocks do show a marked reaction in the years immediately following the aphis peaks of 1929, 1933 and 1939 (zones F and D respectively in Fig. 4), the apparently smaller response to the two later peaks being related to the small proportion of the total stocks then growing in these two areas (centre C) in 1933, there was a clear reaction of the Menai Straits crops in 1934. Degeneration in south Caernarvonshire also shows some correlation with aphis counts at Aberdaron (centre G) in that there is no sign of degeneration until 1935, following on the low peak in 1933. This is succeeded by a temporary improvement in health which, however, again retrogresses after the high aphis peak in 1939.

(*c*) *Effect of topography on crop degeneration.* If it can now be assumed that the correlation between aphis numbers and degeneration is a close one, it must also be accepted that the degree of uniformity with which comparable stocks degenerate in an area

can be used to that extent as a measure of the uniformity of the conditions for aphides in that area. Thus, whilst it is evident that it was only by multiplying the number of 'Entomological Centres' that the very different rates of degeneration of crops in, e.g., Flintshire and Anglesey, became comprehensible, it is equally clear that the lack of uniformity, illustrated by Fig. 4, in the rates of degeneration within some of these geographical areas points to quite local differences in one or more of the conditions necessary for aphis overwintering, migration or breeding. There is, for instance, nothing in the aphis figures at Holyhead to account for the higher rates of degeneration of Anglesey in zones Ac and Ad as compared with Aa and Ab. No simple explanation will cover all the facts but there is little doubt that shelter, in one form or another, is the most obvious difference between 'good' and 'bad' localities for seed potato production. Thus the most successful areas are bleak and completely exposed to strong coastal winds; such are zones Aa and Ab in Anglesey, zone Ca at the tip of the Lleyn peninsula of Caernarvonshire, and the group of hill farms north of Criccieth forming zone Cd. Amongst the less suc-

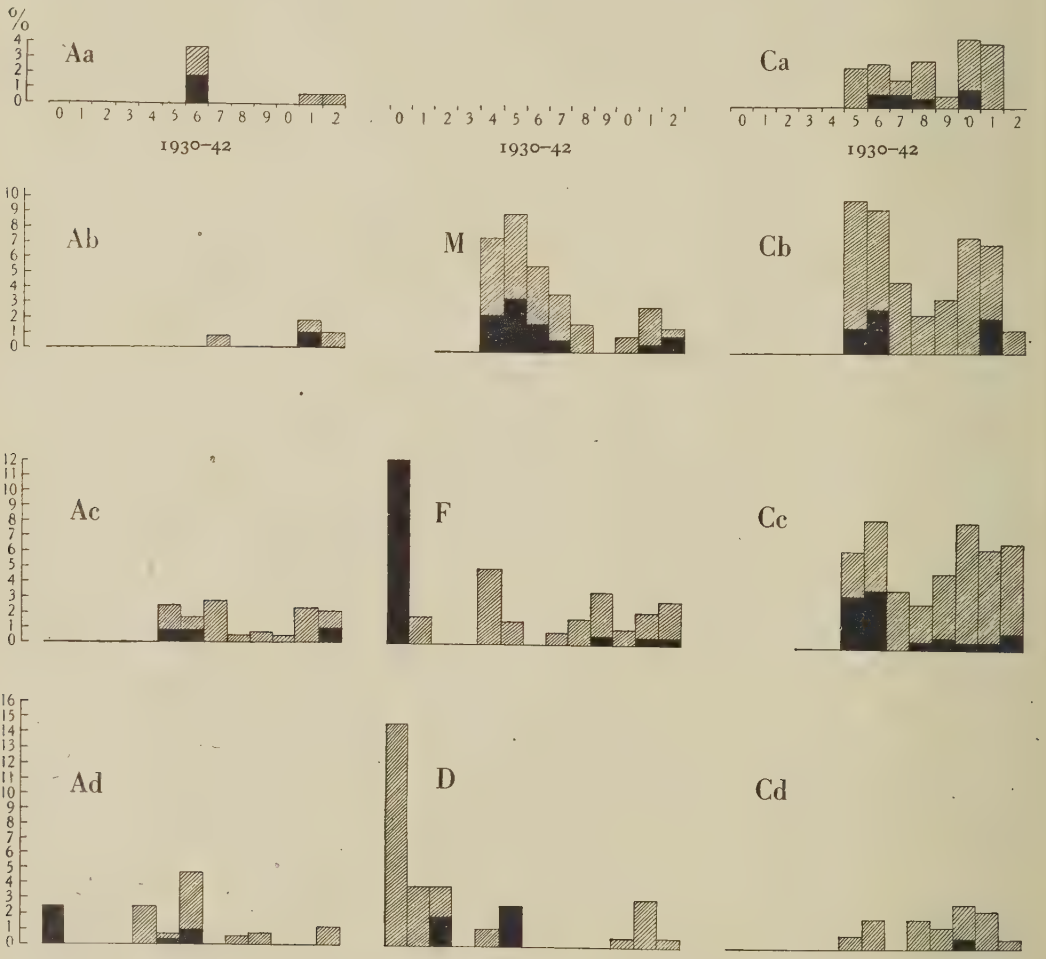

Fig. 4. Rate of degeneration of stocks (1930–42) in different localities or zones.

Aa–d = Anglesey zones.
Ca–d = South Caernarvonshire zones.
M = Menai Straits zone.
F = Zone of arable farms in Flintshire.
D = Zone of hill farms in Denbighshire.

Virus disease expressed as percentage of total stocks under scheme:

= 1–3 % = exceeding 3 %

cessful localities are the Menai Straits zone and the hill farms of Denbighshire. The former is lacking in the extreme bleakness of zones Aa and Ca but is nevertheless exposed to strong south-west winds which sweep through the Straits; it may not be a homogeneous unit and Fig. 4 suggests that zone Ac in Anglesey, which is somewhat sheltered by a low ridge from the south-west (and possibly zone Ad) should be included in zone M on the grounds that the aphis conditions apparently correspond more to those obtaining at the College Farm (centre C) than to those found near Holyhead. The hill farms of Denbigh are not exposed to coastal winds as are those in zone Cd but they cannot be regarded as sheltered; aphis counts on the slopes of the inland valleys, on which most of them are situated, were considerably summarizes the percentage degeneration in eight varieties, and is emphasized in the data with regard to three old-standing varieties in Table 2. The aphis infestation with *M. persicae* during this period at the two centres is shown in Table 3.

It is unfortunate that no record was taken in zone Cb during 1934 when, as can be seen from Table 2 and Fig. 4, considerable transmission of disease occurred. Nevertheless, it seems as justifiable to assume that, in that year, the aphis infestation was heavier in the sheltered zone Cb than in zone Ca, but probably less than was found at the College Farm (centre C), as it is to state that the known heavier infestation on farm C 101 *a* in 1936 affords an explanation of the increase in disease noted in its neighbourhood in 1937. It is indeed made the

TABLE 2. *Virus disease records at three farms in each of the two zones Ca and Cb*

		1933		1934		1935		1936		1937	
Farm	Variety	LR	M	LR	M	LR	M	LR	M	LR	M
C 101 *a**	Sharpe's Express	0·09	0·08	Nil	0·07	0·13	0·18	0·04	0·23	0·27	0·14
	Great Scot	0·15	0·09	Nil	Nil	0·60	0·06	0·57	0·24		—
	Kerr's Pink	0·11	0·05	0·03	0·06	**1·04**	**0·08**	**1·20**	**0·43**		—
C 501 *a*	Sharpe's Express	0·09	Nil	Lifted		0·08	0·01	0·07	0·36	0·62	0·17
	Great Scot	0·17	0·08	0·23	0·16	**1·66**	**0·16**	0·44	0·19	**3·00**	**0·46**
	Kerr's Pink	0·04	0·02	0·13	0·06	0·55	0·03	0·60	0·09	0·60	0·09
C 712 *a*	Sharpe's Express		—	0·06	0·34	0·06	0·07	0·29	0·21	0·37	0·13
	Great Scot		—	0·86	0·13	**1·10**	**0·08**	0·40	0·24		—
	Kerr's Pink		—	0·41	0·11	0·48	0·01	0·16	0·08	**2·42**	**Nil**
C 102 *b**	Sharpe's Express	0·16	0·13	0·11	0·22	0·21	0·21		—		—
	Great Scot	0·16	0·21	0·33	0·11	**0·81**	**0·29**	0·25	Nil	Nil	0·13
	Kerr's Pink	0·20	0·07	0·40	0·07	**2·00**	**0·43**	**4·00**	**Nil**		—
C 602 *b*	Sharpe's Express	0·07	Nil	0·11	0·10	Nil	Nil	0·28	0·56	0·52	0·32
	Great Scot	0·37	0·10	0·47	0·11	**1·42**	**0·07**	**1·21**	**0·09**		—
	Kerr's Pink	0·13	0·07	0·31	0·36	**1·06**	**0·55**	**2·40**	**0·21**		—
C 503 *b*	Sharpe's Express	0·05	0·10	0·31	0·07	0·13	0·24		—		—
	Great Scot	0·08	0·06	0·17	0·08	**3·00**	**Nil**		—		—
	Kerr's Pink	0·03	0·06	0·60	0·28	**6·00**	**Nil**		—		—

Notes: LR = leaf-roll; M = mosaic; — = not grown.

* = 'Entomological Centre'; the other two farms being its nearest neighbours. Infection (LR + M) exceeding 1 % is shown in heavy type.

lower in 1929 and 1933 than at the 'Entomological Centres' near Trelogan and Rhyl, but much higher than elsewhere. As for the other areas where difficulty has been experienced, zone F stretches from the coast at Abergele to Holywell in Flintshire. In the main it is a plateau, rising to some 400 ft. in altitude and within 3 miles from the coast; it has a considerable amount of shelter, in places, in the form of game coverts. The same kind of park land is found in zone Cc on the northern coast of Cardigan Bay; whilst zone Cb is heavily wooded, with deep soil suitable for market gardening, though this is not practised to any extent.

The difference in the rates of degeneration in the adjoining areas of zones Ca and Cb is of particular interest. This difference is clear in Fig. 4 which

TABLE 3. *Index figures of* M. persicae *on farms* C 10 1 *a and* C 10 2 *b*

Centre	1929*	1930	1931	1932	1933
C 101 *a*	24	5	2	4	82
C 102 *b*	208	5	0	5	90

Centre	1934	1935	1936	1937
C 101 *a*	26	12	68	7
C 102 *b*	?	18	26	4

* All species; percentage of *M. persicae* not known.

more probable since it was during the winters of 1931–2 and 1932–3 that W. M. Davies (1934) found the most abundant evidence of the overwintering of *M. persicae* on brassicae.

Factors influencing the effect of aphis numbers on degeneration

(*a*) *Number of disease foci in crop.* The chances of an aphis becoming infective will increase with the number of diseased plants in its immediate vicinity, and on this fact rests the case for early and efficient roguing of such plants from the crop. This has been most thoroughly carried out by Mr Currie and the student Inspectors from the outset. Further, a planting standard of not more than 1 % total virus infection was self-imposed (but was not rigorously adhered to) until it became obligatory in 1936 under

TABLE 4. *Incidence of virus disease in three varieties*, 1928–30

	1928		1929		1930	
Farm	LR	M	LR	M	LR	M
A101*a*	Nil	0·30	0·34	0·07	0·15	0·07
A102*b*	0·26	0·08	0·34	0·04	0·12	0·07
A103*c*	0·12	0·18	0·18	0·08	0·16	0·06
A104*d*	0·15	0·07	0·12	0·04	0·23	0·11
A106*c*	0·21	0·09	0·56	0·02	**1·79**	**0·10**
C101*a*	0·08	0·32	0·12	0·11	0·18	0·05
C103*b*	0·12	0·16	0·12	0·14	0·10	0·04
C104*d*	0·20	0·11	0·08	0·06	0·13	0·07
D101	0·15	0·23	0·23	0·10	**1·55**	**0·03**
F102	0·14	0·17	**1·57**	**0·06**	**33·00**	**Nil**
F103	0·28	0·20	0·30	0·08	**12·90**	**0·09**

LR = leaf-roll, M = mosaic. Infection exceeding 1 % is shown in heavy type.

in 1930, where all species of aphides in 1929 only totalled 154. In view of this surprising result the question is examined further in sixty-eight stocks exposed to very different index counts of *M. persicae* in 1933 and the data are presented in Table 5.

In 1933 only one of these sixty-eight stocks reached 0·6 % total virus infection, the average being 0·21 %; in that year they were exposed to a maximum infestation of *M. persicae* as shown in column 2 of Table 5. They clearly fall into four groups according to their reaction to this and the following season's infestation: (*a*) forty-three stocks maintain their health throughout the 3 years; (*b*) sixteen show a delayed reaction and average less than 2 % in the third year which, however, prevents their use as seed in 1936; (*c*) five stocks exhibit the same delayed reaction, which is so serious that they show nearly thrice the infection permissible for certification; (*d*) four stocks only differ from (*c*) in showing an immediate reaction in 1934, and in their complete breakdown in the following year. Yet (disregarding the two stocks exposed to the high aphis index figure of 900, which merely result in confirming the conclusions reached from Table 4) these very different rates of degeneration occur in stocks with minimal initial disease, rigorously rogued and exposed to not more than 180 *M. persicae* per 100 leaves. Further, the degeneration in the stocks of (*c*) and (*d*), exposed to this moderate infestation, is fully as great as in the two very heavily infested ones. The 'time lag' in the

TABLE 5. *Degeneration as affected by initial disease, aphis numbers and roguing*

Infection exceeding 1 % is shown in heavy type.

1933		1934		1935
Initial disease in 4 varieties less than 1 %	Index figure of *Myzus persicae* at nearest centre	Resultant average infection	Index figure of *M. persicae* at nearest Centre	Final % disease
(*a*) 27 stocks	(*a*) 180	(*a*) 43 stocks averaging 0·31 %	(*a*) 27 stocks (100)	(*a*) 0·42
16 stocks	80		16 stocks (25)	
(*b*) 14 stocks	(*b*) 180	(*b*) 16 stocks averaging 0·41 %	(*b*) 14 stocks (100)	(*b*) **1·75**
2 stocks	80		2 stocks (25)	
(*c*) 1 stock	(*c*) 900	(*c*) 5 stocks averaging 0·50 %	(*c*) 1 stock (500)	(*c*) **7·91**
4 stocks	180		4 stocks (100)	
(*d*) 1 stock	(*d*) 900	(*d*) 4 stocks averaging **1·86** %	(*d*) 1 stock (500)	(*d*) **20·00**
3 stocks	180		3 stocks (100)	

the terms of the Seeds Amendment Act, 1935. Prior to this, the relatively few cases in which infection of between 1–3 % occurred were retained to test the efficacy of roguing, and from Table 4 we see at once that where the aphis index figure in 1929 was of the order of 7–900 (zone F), roguing was quite ineffective in preventing a complete breakdown in health even when only 0·38 % disease was present in the crop.

It will be observed, also, that leaf-roll increased on Farm F102 from 0·14 % in 1928 to 1·57 % in 1929 despite roguing and a very low aphis count in 1928, and the same thing applies to Farm D101, degeneration of all but four of the stocks is also of great interest, and can be seen during the same years in Table 1 and in the lower graph of Fig. 3. An examination of Fig. 4 suggests that the extent of this 'time lag' may have some use in measuring the potentiality of an area for seed-potato production. Thus, the reaction is immediate in zones Ad, M, F, and D; there is a lag of 1 year in the four Caernarvonshire zones and in zone Ac of Anglesey, and this is increased to 2 years in zones Aa and Ab of the latter county.

Evidently, other factors in addition to low initial

disease, low aphis infestation, and roguing, must have operated in order to produce such diverse rates of degeneration. Almost certainly the chief of these factors is the proportion of alatae in the aphis population. It was shown by Davies & Whitehead (1935) that, in 1933, 738 migrating aphides were trapped in 10 days at Trelogan (centre J) as against 20 in the same period at Aberdaron (centre G); whilst in 1934, on the day following the heaviest count of alatae at Trelogan (235 alate *M. persicae* were taken on potatoes, averaging 23 per 100 leaves), not only was none found on 100 leaves examined at Aberdaron but a careful search for 6 hr. failed to yield a single alate *M. persicae*. The aphis count alone, therefore, does not necessarily measure the potential menace to the potato crop, and a rich field is opened for further investigation. The importance of this alatae factor in degeneration may be gauged from the difference in the reaction of the stocks exposed to quite comparably high aphis counts in 1929 and 1939 (Fig. 3). All the three factors of low initial disease, severe roguing, and high aphis numbers in mid-July operated in both years, yet practically none of the stocks reached 3% infection as a result of the 1939 aphis peak, in sharp contrast to the effect of the earlier peak. In 1929 one-third of the stocks were located in Flintshire and Denbighshire, where alatae are believed to be much more common than they are in south Caernarvonshire, in which latter area almost half the 1939 stocks were growing. This difference in numbers of alatae is regarded as largely responsible for the contrast in the reaction of the crops.

(*b*) *The varietal factor in degeneration.* In spite of what has been said as to the close correlation between degeneration and peak populations of *M. persicae*, a study of Fig. 4 leaves the impression that the relationship is much less marked from 1938 onwards, and that far more disease occurs than can be accounted for by the single aphis peak in 1939. The problem is examined further in Fig. 5, in which each column represents one variety and its total height gives the average virus infection of all crops of the variety under the scheme in the year in question.

Starting at the top of Fig. 5 with the four old-established varieties which, in 1935, showed a high average disease content per crop as a result of the 1933 aphis peak, it will be observed that considerable improvement in health had been achieved by 1938, possibly due to rigorous roguing and the strict application of the 'planting standard'. Since then, there has been a definite retrogression in health despite the fact that none of the precautionary measures has been relaxed. This falling off in health is regarded as due in part to the introduction, from about 1935 onwards, of a number of varieties more susceptible to infection than were the older kinds. Some of these varieties were not widely grown or were soon discarded, but the four which have survived up to this date (Red King, Majestic, Arran Pilot and Gladstone) have been included, in the order of their introduction, in Fig. 5. Their responsibility for some part of the general failure in health seems clear from the lower half of Fig. 5 in which the varieties have been segregated; the four old varieties (Sharpe's Express, Great Scot, Arran Banner and Kerr's Pink) remaining more or less stable in contrast to the progressive degeneration shown by the newer introductions.

(*c*) *Type of disease.* One effect of the establishment of new varieties under the scheme has been to alter the relative importance of the two types of expression of virus diseases. Prior to 1938 the overwhelming cause of degeneration was leaf-roll, as illustrated for 1935 in Fig. 5, but later years has seen an increasing importance of the mosaic type of disease. This is purely a matter of varietal susceptibility, for the most recently introduced variety, Gladstone, degenerates almost wholly as a result of leaf-roll infection, whereas this disease plays only an insignificant part in the deterioration of Arran Pilot. The identity of the viruses involved in the 'mosaic' is too big a question to enter upon now but it may be mentioned that the steady increase of 'mosaic' in the variety Red King is of particular interest.

An immediate result of this intrusion of mosaic into the problem is apparently to obscure the clear-cut relationship between aphis numbers and degeneration for, whilst leaf-roll only becomes evident in the year following infection, mosaic sometimes increases in the year of high aphis count and sharply diminishes in the following season (see Great Scot, Majestic, and Arran Pilot for 1939 and 1940 in Fig. 5). This, in turn, has an effect on the value of any health Certificate issued in respect of the crop. For instance, 1938 was a year of low aphis population (Fig. 3) and the column heights in Fig. 5 reflect the average amounts of the two types of virus diseases seen and rogued out by the Inspector, upon which the Certificate was based in that year. There was only minimal virus transmission in 1938 as may be judged by the low incidence of leaf-roll in the progeny. The 1938 Certificate would therefore fairly accurately assess the state of health of seed from the crop in respect of both leaf-roll and mosaic. But 1939 was a peak year for aphides, and the increase in mosaic recorded was probably due to current season infection occurring so early that many plants showed symptoms at the time of inspection. All visibly diseased plants were again removed with the result that, in 1940—which was a low aphis year—the mosaic seen at inspection represented only the small fraction transmitted in 1939 after crop inspection; the leaf-roll, on the other hand, included all transmission occurring before, as well as after, the 1939 disease incidence was recorded. Hence a certificate issued in 1939 was a useful guide to the probable mosaic infection in the seed but grossly under-estimated the incidence of leaf-roll.

Clearly, both the time of onset and duration of

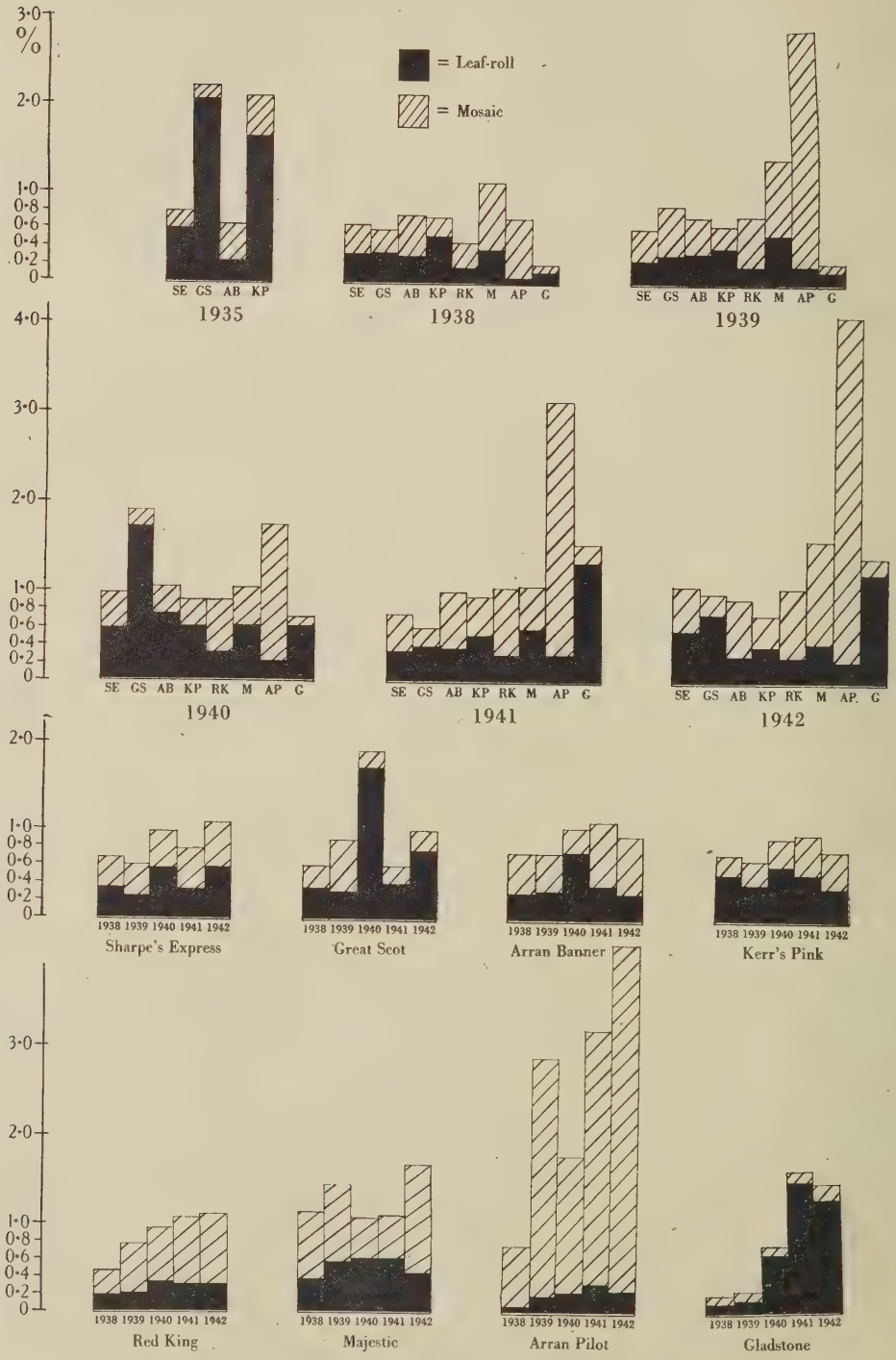

Fig. 5. Effect of variety and type of disease on degeneration.

the aphis infestation must be taken into account in Certification; and here we meet the further complication that, in addition to *M. persicae*, the ability to act as vectors of one or other of the potato viruses has been atttributed to three other species of aphides with very different ecological characteristics. Nothing is known of their relative importance as vectors in the field, but two of these species, *M. pseudosolani* Theob. and *Aphis rhamni* B. d. F. are normally found only in very small numbers on potatoes in North Wales before August; their influence therefore on Certification may only become appreciable when neither of these conditions is normally found; alatae are scarce and the apterous population shows little sign of increase until mid-August, at which time the potato foliage is fully mature or even beginning to die down. But, that sufficiently wide variations in both time of onset and in duration of infestation do occur, even in moderately 'good' districts, is evident from Fig. 6 which has been constructed from data in the late W. M. Davies's field book. This gives the aphis counts on successive dates in 1931, 1932 and 1933, and shows both the progressively earlier onset and the wide differences in duration of attack

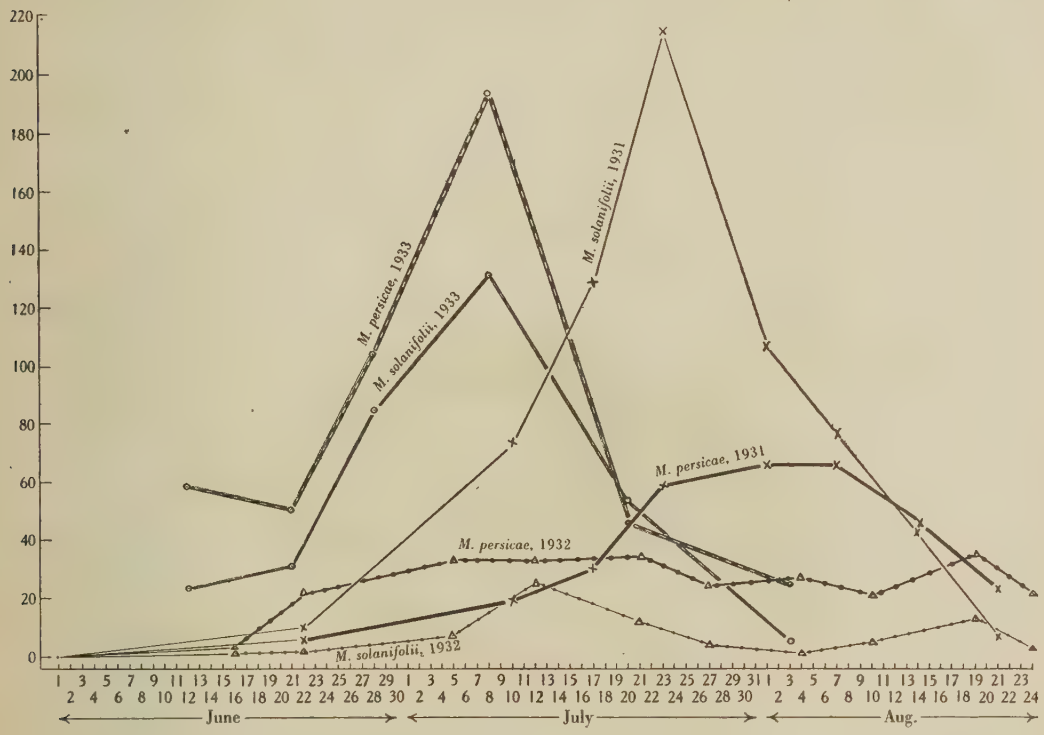

Fig. 6. Aphis infestation on potatoes at Aber (centre C), 1931–3.

the growing season is prolonged and potato blight is subnormal. As regards the third species, *Macrosiphum solanifolii* Ash, Fig. 6 shows that the infestation may exceed in numbers, and coincide in time, with that of *Myzus persicae*; its effect, therefore, as a vector may be added to that of the latter species but, until the value of its contribution can be assessed, it seems better to confine discussion to the efficient vector *M. persicae*.

For mosaic symptoms to develop before the end of July, by which time the crop inspections are usually finished, we must assume, at latest, a heavy June aphis infestation with a proportionately high number of alatae. In the best seed-producing areas over the 3 years at the College Farm, Aber. The 1931 graph discloses a very late attack, with numbers of *M. persicae* only reaching 64 per 100 leaves by 23 July and already falling sharply after the first week in August; whereas the infestation in the following year began to be evident by 22 June and persisted at about half the 1931 level for the rest of the season. Whatever may be the different effect of these infestations on the health of the potato stocks, they must be ascribed to differences in *time* of onset and duration of attack rather than to the *numbers* of aphides involved. On the other hand, migration of alatae in 1933 was exceptionally early and heavy, with the result that the apterous population was

already as high in numbers by 12 June as was ever reached, even in August, during 1931. Hence comparisons of 1933 with 1932 will be largely between aphis numbers, whilst with 1931 the differences must be expressed in terms of both time and numbers.

The evaluation of these many factors under field conditions is an important problem for the future, but some suggestive facts have already emerged. Without doubt, the not uncommon opinion that seasons can be differentiated as 'mosaic' or 'leaf-roll' years is susceptible of explanation on the aphis ecological differences described above; though of course differential transmission of viruses by different aphis species or strains (or even of the same individual at different times) is by no means excluded. Certainly the infestation in both 1932 and 1933 began early enough to give rise to a 'mosaic year', and the fact that it did not do so can be ascribed to the absence of markedly mosaic-susceptible varieties under the scheme; in 1939, however, there was not only a heavy June infestation but an abundance of susceptible varieties such as Majestic and Arran Pilot, with the consequent high mosaic infection of that year followed by a typical 'leaf-roll year' in 1940.

Finally, it is of interest to note that each of the 3 years of early infestation (1932, 1933 and 1939) followed mild winters in which *M. persicae* overwintered in large numbers on brassicae according to Davies (1934) and Jacobs (1941). In this connexion it may be mentioned that a stock of Majestic containing 0·34% mosaic was distributed in the spring of 1939 to three farms; two of these were sheltered, arable, farms in zone Cc in which the conditions were favourable for aphis survival during the winter of 1938–9, whilst the third was a pastoral farm on the bleak hillsides of zone Cd. The crop on the last mentioned farm still showed only 0·32% mosaic at inspection time, whereas the two sheltered crops had already increased their mosaic content to 1·77 and 1·33% respectively. The linkage thus suggested between (*a*) aphis over-wintering, (*b*) early infestation of the potato crop, and (*c*) an appreciable increase in mosaic infection, may be of practical importance in influencing inspection records of disease in market garden (or intensively arable) areas, as opposed to the more pastoral districts; but we must leave it to future work to decide how far all these factors are relevant to a proper understanding of the geographical distribution of leaf-roll and mosaic forms of degeneracy in the British Isles.

Summary and conclusions

The experience of the last 15 years has shown: (1) the value of continuous aphis and virus disease records; it has demonstrated the intimate relationship between them; (2) that a National Survey along these lines would be justified on both scientific and practical grounds; (3) the importance of a high standard of health in the seed planted, without which other precautions such as roguing and relative isolation of crops are ineffective, even with moderate aphis infestations; and (4) that good seed-growing areas are characterized by exposure to strong winds, lack of facilities for aphis over-wintering, low and late aphis attacks with a corresponding scarcity of alatae.

From these facts it is concluded that, failing effective field control of aphides:

(*a*) Class I Certificates should not be automatic over large geographical areas, but should be confined to districts conforming to the conditions described under (4) above.

(*b*) A parentage of at least T.S. (A) standard (i.e. less than 1% total virus disease) from Class I *areas* only, should be required before a stock is inspected for certification.

(*c*) Certification of stocks in areas normally with early and high aphis counts has no scientific justification; such districts would gradually be delimited as the survey suggested under (2) progressed. Conversely, it is believed that many potentially good areas would be disclosed.

(*d*) Deficiencies in good seed-stocks available for sale should be made up by encouraging the planting of small ware or chats from the best seed-growing districts, rather than by the issue of Certificates of doubtful value to stocks in other, less suitable localities.

(*e*) As a war emergency measure for reducing transport, County War Agricultural Committees should be encouraged to plant good seed in selected areas for use as seed in the county. Unsuitable areas would not survive post-war competition but others would find a permanent place in the industry.

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V. Ecology of potato aphides in North Wales

By I. Thomas, Ph.D. and F. H. Jacob, M.Sc.
School of Agriculture, University College of North Wales, Bangor

(With 4 Text-figures)

Work on the ecology of potato aphides in North Wales was started by Walton as long ago as 1925. Since that time the virus aspect has been studied in detail by Whitehead, and the problems of aphis ecology have been the subject of a series of papers by Maldwyn Davies. Davies's main conclusions were as follows:

(1) The optimum conditions for flight of *Myzus persicae* Sulz. are a temperature above 70° F., a low relative humidity, and a wind velocity below 4 miles/hr.

(2) These conditions seldom occur in the western parts of the North Wales province where the seed-potato-growing industry has been developed.

(3) The maximum infestation of *M. persicae* generally occurs in mid-July and the numbers per 100 leaves at this time of the year may be regarded as an index figure for any potato crop in a particular district. In seed-producing districts the index figure is generally well below 100.

(4) The most important mode of over-wintering of *M. persicae* is on winter Brassicae particularly savoys.

Since Maldwyn Davies's publications three papers having a bearing on potato aphides have been published at Bangor, one by Thomas & Vevai on aphis migration, one by Jacob on the overwintering of *M. persicae*, and one by Vevai on *Aphidius matricariae* Hal., a Braconid parasite of *Myzus persicae*. The conclusions arrived at by Jacob in his study of overwintering were as follows:

(1) It is not likely that *M. persicae* overwintered in North Wales on field Brassicae during the winter of 1937–8.

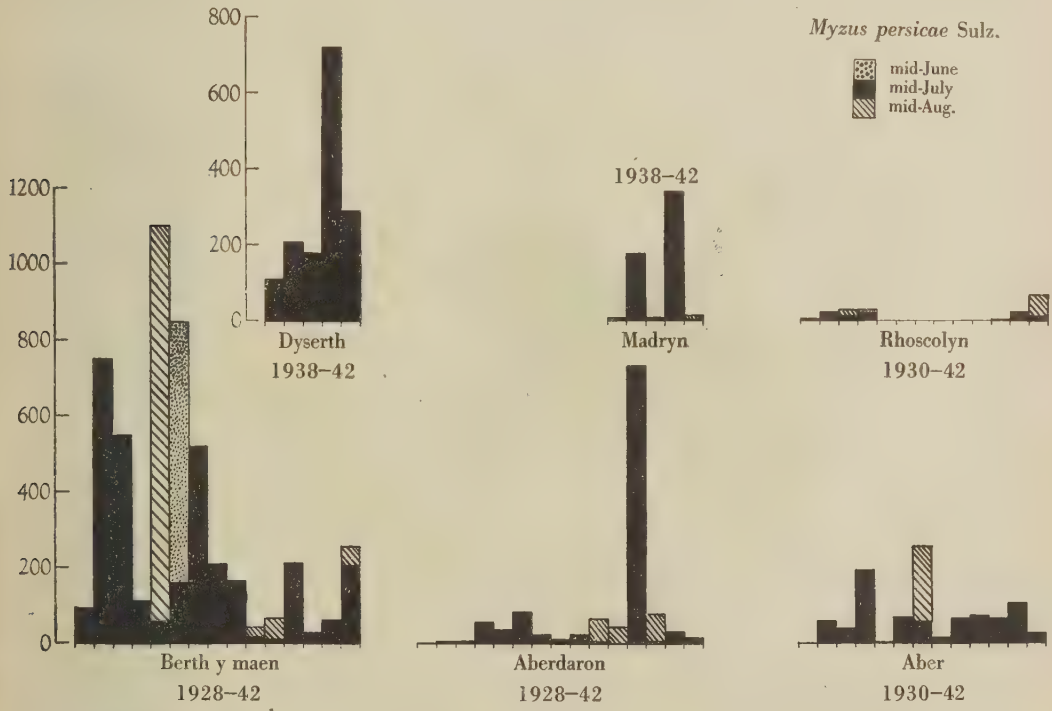

Fig. 1. Index figures for *M. persicae* Sulz. at a number of North Wales centres; Berth y maen and Dyserth, Flintshire; Aberdaron, south-west Caernarvonshire; Madryn near Pwllheli, Caernarvonshire; Aber near Bangor and Rhoscolyn, north-west Anglesey.

(2) A considerable number of *M. persicae* overwintered both under field and garden conditions in the winter of 1938–9: savoys were the favourite host plant.

(3) As far as could be ascertained no *M. persicae* overwintered out of doors during the winter of 1939–40.

(4) Peaches are of minor importance as a winter host plant in North Wales, but glasshouses form a reservoir from which numerous *M. persicae* may migrate in spring and early summer.

(5) It seems probable that years of high infestations follow winters when widespread hibernation still the number of aphides per 100 leaves. We have tried a method suggested by Ris Lambers which consists of beating a number of plants at random through the crop. The following are some figures obtained by the counting and beating methods in the same crop:

	Aulacorthum solani Kalt. = *M. pseudosolani* Theob.	*M. persicae*	*M. solanifolii* Ash.	*A. rhamni* Boyer
By counting 150 leaves	3	97	19	113
By beating 50 plants	30	55	50	2

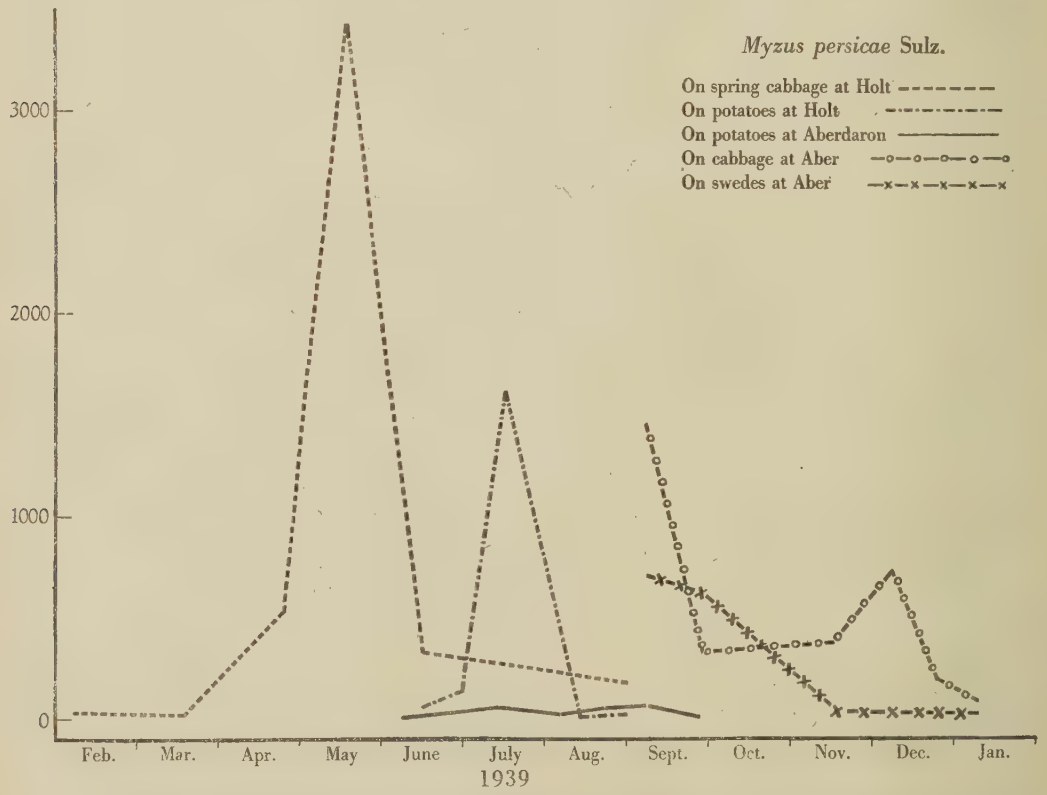

Fig. 2. 1939 populations (per 100 leaves) of *M. persicae* Sulz. on spring cabbage at Holt, east Denbighshire and on potatoes at Holt and Aberdaron, south-west Caernarvonshire. 1930–40 winter populations of *M. persicae* on cabbages and swedes at Aber near Bangor.

on Brassicae has occurred, and vice versa, years of low infestations follow years when a few or no *M. persicae* overwinter on Brassicae.

The population counts started by Davies have been continued until the present but a slight modification of his method of counting has been used. Instead of taking 100 leaves at random from the crop we now take fifty plants at random and select a top, middle and bottom leaf. Our index figure is

It is clear that beating does not give results comparable to the counting of the aphides on 100 leaves. It would possibly give more accurate figures for *M. pseudosolani*, a species which very readily falls off a leaf when disturbed. On the other hand, it seriously under-estimates the population of *Aphis rhamni*, a species which occurs largely on the lower leaves and is difficult to dislodge by the ordinary beating methods.

The index figures for *Myzus persicae* for a number of centres in North Wales are shown graphically in Fig. 1. Where the August figure is higher than the July figure it is represented by the diagonal lines; where the June figure is the highest for the year it is shown by stippling. In 2 years only is the August or June figure very much higher than the mid-July figure. On the whole the index figures in the good seed potato growing districts are very much lower than in the eastern parts of the province as shown by Maldwyn Davies. The one exception occurred at Aberdaron in 1939 where an index figure of over 700 was recorded. In this year the weather during May and June throughout North Wales was abnormally fine and sunny. At Pwllheli for instance, the average relative humidity for June was 76·8 compared with the usual value of over 80, and the total sunshine for the month was 263 hr., over 100 hr. more than is sometimes recorded in this district, the average for the 10 years up to and including 1939 being 206 hr. There were seven consecutive days early in June when conditions were favourable for migration, and at Aber the record number of 1205 aphides was recorded on one day in this period. Conditions beyond Pwllheli were even more favourable because the abnormally fine conditions amounted to a serious drought and cattle were turned into the corn fields.

Another important point illustrated in Fig. 1 is the difference between the index figure at two centres very near together in Flintshire. The figures for Dyserth were taken at a farm where early potatoes are grown for the market. These come through the ground earlier, are infested earlier by *M. persicae*, and the index figures by July are considerably higher than in the case of the main crop potatoes. The difference was also noted on early and late crops at Berthymaen in 1939.

Fig. 2 shows the population of *M. persicae* on spring cabbages at Holt followed by the population on potatoes in the same district. Large numbers of aphides with wing buds and alatae were produced on the cabbages in mid-May and alatae were found on the potatoes in early June. These bred rapidly to give the mid-July peak index figure of about 1500. The population at Aber is also recorded: it remained below 100 per 100 leaves throughout the season.

The other two curves show the normal trend of the population on Brassicae from September to January. From about 1500 per 100 leaves it decreases to less than 100 by mid-January. In some seasons *M. persicae* may persist on Brassicae whereas in others it is killed.

We have in recent years paid some attention to the biology of *M. solanifolii* Ash. So far we have not found this aphis on rose which is commonly

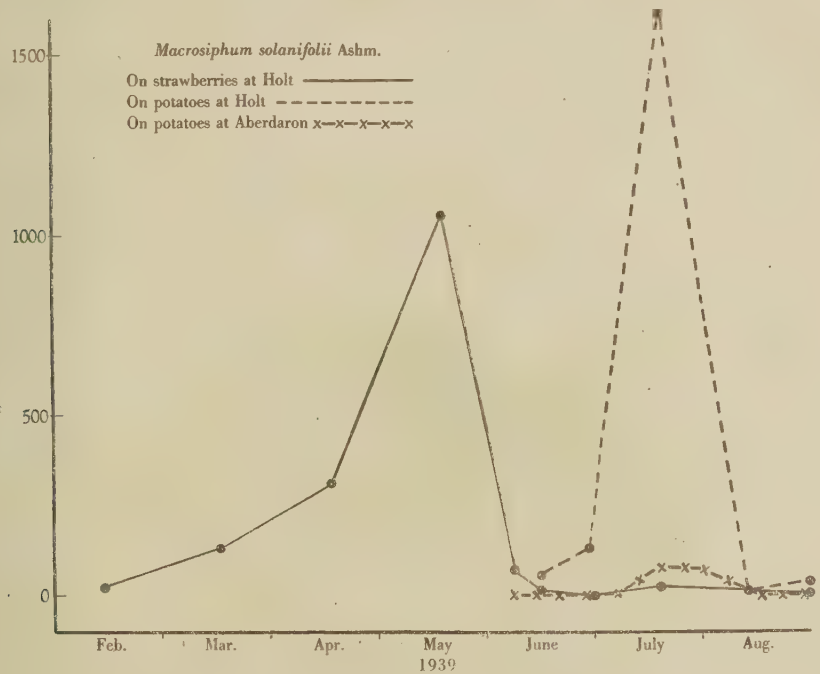

Fig. 3. 1939 populations (per 100 leaves) of *Macrosiphum solanifolii* Ash. on strawberries and potatoes at Holt, east Denbighshire (strawberry figures, × 5).

supposed to be its overwintering host plant. On the other hand, we have found it overwintering on strawberries in considerable numbers. Fig. 3 is a graphical representation of what occurred on strawberries at Holt in 1939 (so that the populations for strawberries correspond more closely to those for potatoes per unit area of leaf, the figures for the former have been multiplied by 5). A few apterae may overwinter as such but eggs are also laid on strawberries. These hatch in late March or early April and the population increases at first slowly and then rapidly to give a peak population about mid-May. Large numbers of alatae are produced about this time and these migrate to other plants and are generally found on potatoes in late May or early June soon after they come through the ground. At Holt there was an index figure of over 1500 compared with less than 100 at Aberdaron. This is the normal state of affairs in North Wales. The index for *M. solanifolii* is generally above but may be below that of *M. persicae*. It is well known that the habits of the aphides are different, the former often occurs in large numbers in the flowerheads and the latter on the middle or lower leaves. Like *M. persicae*, *M. solanifolii* also overwinters in large numbers in glasshouses.

Since 1938 we have made a more detailed study of the life history of *Aphis rhamni*. Its overwintering host plants are recorded in the literature as *Rhamnus frangula* and *R. catharticus*. Although records of the occurrence of the latter are given for North Wales specimens were found only on the Flintshire and Cheshire border—*R. frangula* can be more readily found but we found no eggs of *Aphis rhamni* on this plant. At Holt and Farndon *A. rhamni* migrates from buckthorn to potatoes in early June soon after the plants come through the ground. The population increases at first slowly and later rapidly to give a peak generally about 3 weeks later than that for *Myzus persicae*, i.e. about the end of July to mid-August. We have also found this aphis on nasturtiums and *Symphytum officinale* from which plant it was originally described. According to Ris Lambers it should now be called *Doralis symphyti* Schr.

The numbers on potatoes vary greatly in different

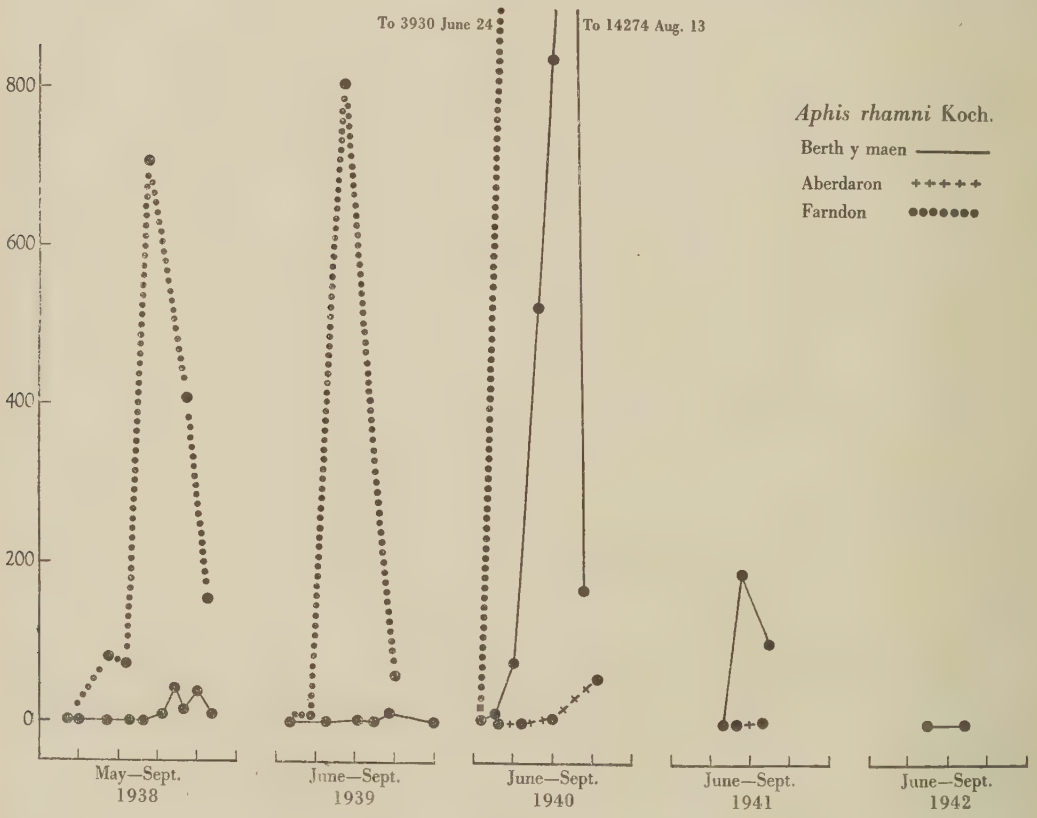

Fig. 4. 1938–42 populations (per 100 leaves) of *Aphis rhamni* Boyer at three North Wales centres; Farndon, Cheshire-Denbighshire border; Berth y maen near Rhyl, Flintshire; and Aberdaron, south-west Caernarvonshire.

years. In 1938 there were at Farndon, just outside the eastern border of the North Wales province, about 700 per 100 leaves; in 1939 about 800 per 100 leaves, whilst in 1940 there were 3930 per 100 leaves as early as 24 June. No records were taken at this centre in 1941 and 1942. At the Flintshire centre, not far from Rhyl, the figures were very low in 1938 and 1939 but on 13 August 1940 the count was 14,274 per 100 leaves. The peak figure was only about 200 in 1941 and less than 10 in 1942 (Fig. 4).

At Aberdaron in the south-west tip of the Caernarvonshire Peninsula none was found in 1938 and 1939 but in 1940 *Aphis rhamni* reached this point and bred slowly to give a very low peak population in early September. In 1941 also a few were recorded at this centre. In unpublished work Joyce noted similar variations in the years 1936 and 1937. He says: 'In 1936, the numbers of *Aphis rhamni* were very variable. In the Holt district of Cheshire, it was the dominant species in mid-July outnumbering *Myzus persicae* by nearly 3:1. Similarly, in one crop at Aberdaron, *Aphis rhamni* was dominant, again outnumbering *Myzus persicae* by nearly 3:1, while, in an adjacent crop, *M. persicae* outnumbered *Aphis rhamni* by the same degree. In 1937, *A. rhamni* was recorded at only the eastern centres, and at Aber; neither *Myzus persicae* nor *Aphis rhamni* being recorded in the Lleyn peninsula in 1937. *A. rhamni* was dominant in August in both Trelogan and in Ty Mawr. It is generally the case, both in 1937 and in previous years, that *Aphis rhamni* is recorded later than other species of potato aphides, and reaches its maximum later in the season.'

From observations in North Wales over a number of years it is evident that the potatoes nearest buckthorn, the overwintering host plant, are colonized in early June. In some years very high populations rapidly breed and there is widespread distribution by alatae to other potato crops. When the early populations in the eastern districts are very high the species eventually migrates right across North Wales to reach the most westerly point. In seasons of low population in the east *A. rhamni* seldom migrates as far west even as Bangor.

Much more detailed information on the biology of *A. rhamni* is available and will be published at a later date.

VI. The life history of *Aphis* (*Doralis*) *rhamni* B.d.F. in eastern England

By J. P. Doncaster, B.A., *Research Officer, Agricultural Research Council, Rothamsted Experimental Station, Harpenden, Herts.*

In recent years, the important part played by *Myzus persicae* in the dissemination of potato viruses has led to a considerable amount of work on the life and habits of this species. The results of much of this work have been published, and we have been given further information on the subject to-day. Lately, however, it has been shown that at least one other species of aphis commonly infesting potatoes, *Aphis rhamni*, is nearly as efficient a vector of potato virus *Y* as is *Myzus persicae*. Although a number of descriptions of this aphis have been published, little information is available concerning its habits of life in this country. During the course of a potato aphis survey in eastern England over the past three summers, I have been able to observe the various stages in its life cycle in some detail; and while realizing that the results from only three seasons' work are far from adequate grounds on which to base any final conclusions, I consider that the facts so far ascertained, and their bearing on the problem of virus transmission, are of sufficient importance to be worth presenting at this stage.

Aphis (*Doralis*) *rhamni* is the commonest aphis found infesting potatoes in eastern and southern England. It is small—about half the size of *Myzus persicae*—and the wingless forms are pale yellow or yellow-green. Unlike *M. persicae* its movements are sluggish, and it tends to congregate in dense colonies on the undersides of the leaves on which it is feeding.

Its winter host plants are the two British species of buckthorn, *Rhamnus catharticus* and *R. frangula*, on which it lays its overwintering eggs. The eggs hatch during the latter half of April, and a brood of wingless females develop, which give rise to two or more generations of aphides, some of which are winged. These are the spring migrants, which leave the buckthorn and fly in search of potatoes, their summer host plants. Although buckthorn is not a common shrub, it occurs in most parts of England, and as a potential source of *Aphis rhamni* its scarcity is offset by the large numbers of aphides which may develop on it. In the spring of 1941, and again this year, some buckthorns were found so heavily infested that severe leaf distortion was apparent over most of the tree, and single leaves were observed bearing a hundred or more developing migrants. Assuming that the average number of developing migrants is no more than twenty per leaf, a single small buckthorn, of the type commonly found in hedgerows, would be capable of producing 300,000 migrants, or one migrant for every plant in 20 acres of potatoes.

An initial infestation on potatoes of 100% is, of course, rarely, if ever, met with in nature, the migrants nearly always becoming widely dispersed during the course of their migration. A proper study of the spring migration of *A. rhamni* involves the whole question of aphis flight, a subject on which

much work has been done, but which is still incompletely understood. There appear, however, to be two main types of aphis flight; the first an active, or purposeful flight, which can be undertaken only during favourable conditions when the air is still and dry, and in which the aphis flies under its own power for short distances; and the second, a passive flight, in which the aphis is caught up and carried by the wind, sometimes at considerable heights, and often for distances of many miles. It seems probable that passive flight plays the greater part in transporting *A. rhamni* from its winter to its summer feeding grounds. This assumption is supported by the fact that potato crops situated near buckthorn trees do not become appreciably more heavily infested than crops at considerable distances from them. The aphides, however, will have to resort to a certain amount of active flight at the end of their journey in order to find their way on to suitable host plants. The rarity with which *A. rhamni* is found on any plants other than potato points to the fact that they do exercise a considerable degree of active selection at this stage. The spring migrants leave the buckthorn during June, and are found colonizing potatoes during the latter part of that month, and during the first half of July. By this time the early varieties of potato are well advanced, and most of the main crops are above ground. By mid-July all aphides have left the buckthorns.

The behaviour of the spring migrant after its arrival on the potato crop is still somewhat obscure, largely owing to the difficulty of making detailed observations on individual aphides in the field. Certain deductions can, however, be made from the results of the field sampling methods used in the aphis survey. These suggest that, as has already been established in the case of *Myzus persicae*, the migrants do not settle permanently on one plant, but alight to feed for a short time only, during which young may be produced, after which they fly to other plants where this process is repeated. Evidence in support of this assumption is afforded by the frequency with which isolated newborn young are found, scattered in ones and twos on separate plants throughout the crop; by the rapidity with which fields become colonized; by the random distribution of infested plants; and by the infrequency with which the migrants themselves are seen. This restlessness on the part of the spring migrants may be of great importance to the question of the spread of virus *Y*.

The young deposited by the spring migrants multiply rapidly and give rise to colonies of wingless females, which tend to remain concentrated on single plants or small groups of plants. For this reason populations of *Aphis rhamni*, early in the season, are never of a uniform density throughout. As the spring migration covers a period of some weeks, and as the progeny of the migrants multiply at a more or less uniform rate, there is formed a succession of developing centres of infestation. This state of affairs is less pronounced in the case of *Myzus persicae*, whose wingless forms are more active, and tend to disperse throughout the crop, rather than to remain in colonies.

Despite the apparent reluctance of the wingless forms of *Aphis rhamni* to change their position, they are often forced to do so by overcrowding, or by the ageing and withering of the leaves on which they are feeding. Observations on individual infestations showed that some of the aphides dislodged in this way crawled upwards to fresh leaves on the same plant, while others found their way on to neighbouring plants, and there was evidence to show that movements of wingless aphides were more likely to take place between plants in the same row, rather than between plants in different rows. It seems probable also that any violent disturbance of the plant by wind or rain, or by the passage of a spraying machine, for instance, may cause such movement to occur.

Certain it is that, under favourable conditions, once *A. rhamni* has become established, the infestation spreads throughout the crop with remarkable rapidity. On two Fenland fields sampled in 1942 *A. rhamni* was first recorded on 15 June, when from 7 to 13% of the plants sampled bore aphides. A fortnight later 40% of the plants were infested. This rapid spread of the infestation throughout the crop may be attributed in part to the movements of the wingless aphides, but the process is completed by the development of a brood of winged 'dispersal migrants' from the colonies within the crop. The frequency with which the dispersal migrants are encountered during sampling suggests that their flights are restricted, and it seems that many of them colonize plants in the same field, while others fly to neighbouring crops. It is not uncommon to find a field which has escaped the attentions of the spring migrants becoming colonized by dispersal migrants from an early-infested field in the vicinity.

A demonstration of the activities of the dispersal migrants was afforded during the course of an experiment designed to test the effect of nicotine fumigation in controlling aphis infestations in the field. A crop of King Edward potatoes was divided into four blocks, two of which were fumigated and two left as controls. The nicotine vapour was applied on 13 July, by which time the infestation of *A. rhamni* had reached a density of from 3000 to 5000 per 100 leaves. On the following day no live aphides were found on the treated blocks. Subsequent aphis counts, however, showed that recolonization of the treated blocks by winged aphides from other parts of the field took place rapidly, so that by 10 Aug. the level of infestation on the fumigated blocks had equalled or even exceeded that on the controls. Aphis numbers on the treated blocks continued to increase and reached a maximum of about 1900 per 100 leaves on 24 Aug.

The dispersal migrants first appear at about the time when the colonies of wingless aphides are approaching their maximum density. As these colonies develop independently, the plants first colonized by the spring migrants are usually the first to produce the dispersal forms. As successive colonies reach maturity, more and more migrants are produced, and the peak period for the production of migrants usually coincides with the maximum for the infestation as a whole.

In 1942, when climatic conditions seemed particularly favourable for the development of *A. rhamni*, peak infestations were recorded in Lincolnshire during the second week in August, and about a week earlier in Bedfordshire and Kent, that is, from 6 to 8 weeks after the initial infestation. On every one of eleven fields surveyed in these areas, 100% of the plants sampled were infested by the time the infestations had reached their maximum; and on three of these fields over 90% of the leaves were found bearing aphides. Peak figures varied from 900 to 14,000 aphides per 100 leaves, and the fields on which the highest peak figures were recorded were those which had become infested first. Fields infested later, though they reached their peak figures at about the same time as the others, never attained the same degree of infestation density.

The degree of uniformity, both in the time of the maximum infestations and in their density, is in marked contrast to the results of the surveys carried out in the two previous seasons. In 1941, especially, infestations were extremely variable, peak figures ranging from only 70 per 100 leaves to just over 4000 in the Lincolnshire fens, and the dates at which the peaks were reached occurred from 2 to 4 weeks later than those recorded in 1942. The exceptionally high rainfall and consequent low temperatures during July and August of 1941 were probably the main cause of the delay in development of the colonies.

I have been quite unable to find any correlation between the density of *A. rhamni* populations and the locality of the crop. Samples made on fields in the same districts, even on adjacent fields on the same farm, may show widely divergent infestation figures, both as regards density and the length of the development period. Conversely, widely separated fields, on different soils and at different altitudes, may yield very similar infestation figures. Nor does there appear to be any correlation between aphis population and potato variety, with the possible exception of King Edward, which is frequently, though not always, found to be more heavily infested by *A. rhamni* than other main crop varieties.

The growth and development of aphides are influenced to a considerable extent by climatic conditions. Both the rate of growth and the rate of reproduction are increased by high temperatures, and the effects of humidity and wind speed on aphis migration have already been mentioned. It is sometimes stated that heavy rainfall has the effect of reducing aphis numbers by washing the insects off the plants: I have been unable to find any evidence in support of this. After the frequent heavy rainstorms in July and August of 1941, there was no appreciable diminution of aphis infestation on the fields under observation.

Apart from climatic factors, the activities of parasites and predators play an important part in controlling aphis populations. Soon after the infestations have become established, hymenopterous parasites of the family Braconidae appear, together with the predacious larvae of syrphid flies, lace-wing flies, and coccinellid beetles. These live among the aphis colonies throughout the summer, and although their attacks may be severe locally, particularly where the colonies are dense, they do not succeed in preventing the development of the infestations as a whole. The peak period of an infestation is followed by a sharp decline in aphis numbers, particularly when the infestation has been heavy. This decline is caused primarily by the departure of the autumn migrants, which fly back to the buckthorns at the end of the season, but it is undoubtedly accelerated by the activities of the parasites and predators, which are naturally more numerous on fields where the aphis infestations have been dense.

The autumn migrant is indistinguishable morphologically from the dispersal migrant, and it is impossible to determine accurately the point at which autumn migrants first appear. Insect trap records, combined with field sampling data, show that the production of winged migrants from within the potato crop is a more or less continuous process from the time at which the first colonies reach maturity about mid-July. In 1941, small numbers of migrants appeared on the traps during the period when the populations were multiplying on potatoes, but these numbers increased rapidly shortly after the peak infestation had been passed. This increase corresponded with the arrival of migrants on the buckthorn. Having arrived there, the migrants give birth to young which develop into wingless egg-laying females. In the meantime, from among the remnants of the colonies on potatoes, winged males develop and migrate to the buckthorns, arriving by about the time that the egg-laying females are mature. Mating takes place on the tree, and egg-laying commences towards the end of September.

In 1941 the autumn migration commenced about 18 Aug., and continued for about a month. An aphis count on a buckthorn in Spalding gave 278 migrants, and 2600 nymphs, per 100 leaves on 5 Sept.; a week later, on another tree, the numbers of migrants were less, but the young numbered 13,000 per 100 leaves. On 24 Sept. egg-laying females, many of which were mature and ovipositing, numbered up to 100,000 per 100 leaves. By this time most of the migrants were dead, and some were found to have been parasitized. Males occurred in trap catches between 12 and 28 Sept.

In 1942, however, conditions were very different. Large numbers of migrants left the potatoes during August, and the first arrivals on buckthorn were noted on 12 Aug., about a week earlier than in 1941. The colonies on buckthorn multiplied, and on 26 Aug. an aphis count near Spalding gave approximately 300 migrants, and 2000 nymphs, per 100 leaves—figures very similar to those recorded on 5 Sept. 1941. After that, however, the colonies rapidly declined and repeated searches on buckthorns in seven different localities during September failed to reveal more than a few isolated oviparae and one or two migrants.

There seems little doubt that the activities of predators were largely responsible for this decline in aphis population on the winter hosts. The occurrence in 1942 of very large numbers of aphides of all species (with the noteworthy exception of *Myzus persicae*) seems to have resulted in a corresponding increase in the numbers of predators. Adult coccinellid beetles were found on many of the buckthorn trees, and were noticed in large numbers feeding on colonies of the aphis *Anoecia corni*, a common pest of dogwood which is often found growing in association with buckthorn.

This story is still not yet complete. On a buckthorn tree in Harpenden the autumn migrants were still arriving on 9 Oct. 1942, and a count of 100 leaves on 7 Oct. gave four winged aphides and forty-seven developing oviparae; a slight increase on the numbers recorded the previous week, but representing a very small fraction of those recorded a year ago at this time.

To sum up: the life cycle of *Aphis rhamni* is better defined in its essential features than is that of *Myzus persicae*. Its host range is small: whereas the potato plant is only one of a large and varied range of food plants which compose the menu of *M. persicae*, it is the only summer host plant on which *Aphis rhamni* is found in any numbers. In the winter, *Myzus persicae*, in addition to laying winter eggs on peach trees, may be found living and reproducing on a variety of glasshouse plants, and, in favourable conditions, even out of doors. *Aphis rhamni* lays its eggs on buckthorn, and has no alternative means of passing the winter. The importance of *Myzus persicae* as a vector of potato viruses is already established, and there seems little doubt that its polyphagous habits and the greater activity of its wingless forms are largely responsible for its success. The potentialities of *Aphis rhamni* as a vector of potato viruses in the field remain to be explored, and it is hoped that these studies on its life and habits may form a prelude to such investigations.

VII. The spread of potato virus diseases in the field

By P. H. Gregory, Ph.D., *Research Officer, Agricultural Research Council, Rothamsted Experimental Station, Harpenden, Herts*

Hitherto, investigations on the spread of viruses in the field have been confined mainly to Scotland, Ireland and the recognized seed-growing areas of England and Wales. In the ware-growing districts of England, however, the seed-size progeny of a crop is often used for planting again at least once, and sometimes twice or more. The limiting factor to the number of times seed can profitably be grown is set by the increase within the stock of the insect-borne virus diseases, leaf-roll and severe mosaic (virus *Y*). Such increase is very variable and depends to some extent on factors within the control of the farmer. The present work was begun in 1940 in an endeavour to improve our knowledge of all factors influencing the rate of degeneration of health of potato stocks in the ware-growing districts of England.

In a study of the spread of virus diseases *within* potato fields the co-operation of Advisory Mycologists and others was obtained, and comparative tests were carried out in various parts of the country. In the 1940 and 1941 seasons it became obvious that the amount of spread around secondary leaf-roll plants varied enormously between different fields, even between fields only a few miles apart. One task of the investigation is to learn to recognize the conditions under which serious spread of virus takes place. Samples taken from experimental fields have to be grown the following year before they can be assessed for the presence of virus diseases, and so, with data from only two seasons yet available, it is premature to attempt to generalize on the field spread of viruses. However, from records obtained on the progenies of crops studied in 1941 it is possible to distinguish several components of the progressive increase of virus during the season in the English lowlands. Periodical sampling of Majestic potato crops showed that virus *Y* had infected a small percentage of the plants and reached the tubers by the middle or end of July, and that there was little if any subsequent increase in the percentage of plants infected with this virus. A small percentage of leaf-roll infections was also noted in July, and while on some fields this percentage did not increase, on others, after a stationary period, the percentage increased steadily during the rest of the season. From periodical counts of aphides made on some of these fields by Mr J. P. Doncaster it appears probable that virus *Y* was spread by the winged spring migrants of *Aphis rhamni*, which reached the potato fields in

late June or early July. This would be in agreement with Dr Kassanis's demonstration that this species is an efficient vector of virus *Y*, and with Dr Marion Watson's evidence that starvation and short feeding periods increase the efficiency of vectors of this virus. On many fields a high population of *Myzus persicae* is associated with a large increase of leaf-roll, but equally it is possible to find large populations of *M. persicae* or *Aphis rhamni* with very little spread from leaf-roll or virus *Y* infectors to nearby plants. Or again there can be a large spread of leaf-roll with low *Myzus persicae* and moderate *Aphis rhamni* counts. Evidently the identification and counting of aphides on a crop will not by itself enable predictions to be made on the health of the progeny. Local factors controlling aphis movement must also be important. For example, it was noticed that on several fields where the haulm remained small and compact the percentage of plants affected with leaf-roll did not increase after July, whereas on certain other fields remarkable for the immense growth of haulm (6–7 ft. and weighing over 25 tons/acre) the largest increases of leaf-roll in August and early September were recorded. It seems likely that a sprawling and densely interlacing foliage would greatly facilitate the movement of wandering aphides, such as the apterae of *Myzus persicae*, from plant to plant.

One precaution that can be taken against the rapid degeneration of potato stocks is to ensure that healthy seed shall be planted as far as possible in isolation from badly diseased stocks. The spread of virus *between* fields has accordingly received attention. It has been found that with increasing distance from a badly diseased stock the amount of virus infection passing over to a healthy stock falls off steeply at first but that the curve then flattens. This infection gradient is receiving further study.

VIII. Potato breeding for virus resistance

By George Cockerham, Ph.D.
The Scottish Plant Breeding Station, Edinburgh

Cultivated varieties of the potato have long been known to differ widely both in ability to withstand virus diseases in the field and also in response to virus infection in the laboratory. In view of the possibility that factors for resistance are concerned in ordering these differences it is pertinent to inquire into their nature and to examine them from the aspect of their possible utilization in virus control.

Tolerance

A general form of difference between varieties, common to all potato viruses, is that of variation in degree of tolerance after infection has been accomplished. The factors determining these variations have been examined by submitting selected seedling progenies, mainly of inbred parentage, to infection with viruses *X* and *Y* and the leaf-roll virus. Segregation for intensity of effect occurred in all progenies but no seedling, however little affected, failed to accept the virus to which it was exposed. Selection for high degrees of tolerance resulted in increased proportions of highly tolerant seedlings in subsequent progenies but all were susceptible to infection. These results are indicative of hereditary factors capable of modifying symptom expression, but they provide no evidence that factors for resistance are concerned for it is clear that the maximum effect obtainable in segregation is a high degree of tolerance whereby the host plant is enabled fully to support the virus but not to resist it.

A high degree of tolerance is in itself of potential value. The disadvantages of so-called carrier varieties are that they serve as reservoirs of virus from which infection may spread and that they are particularly susceptible to complex diseases upon infection with a second virus. The former would be of no serious consequence were all varieties highly tolerant, however, and the latter would be guarded against if protection were afforded against infection with possible complementary viruses. There is evidence that some form of protection can be obtained against most potato viruses of economic importance in the British Isles, with the notable exception, as yet, of virus *Y*. The introduction of potato varieties combining a high degree of tolerance of this latter virus with protection against viruses *X*, *A* and leaf-roll may yet prove, therefore, to be the key to the control of severe virus diseases in this country.

Field immunity

A form of protection against virus *X* has been found in varieties which react to positive infection with symptoms of top necrosis (Cockerham, 1937, 1939). This protection, which is brought about by a hypersensitive reaction of the plant tissue confining the virus within a necrotic barrier at the site of entry, is highly efficient and varieties endowed with it have been described as field immune. Hypersensitive reaction has been found to give effective protection against viruses *A*, *B* and *C* as well as against virus *X* and varieties which express top necrosis upon positive infection with these viruses are consequently field immune from them.

Top necrosis is readily brought about by graft

infection and by using this character as an indicator Cadman (1942) showed that field immunity from virus *X* is dependent for its expression upon the presence of a single dominant gene and that a distinct gene, also dominant, controls field immunity from virus *A*. Evidence of linkage between these two genes was given in the same paper. It has been established separately (unpublished data) that two further, unrelated, dominant genes control field immunity from viruses *B* and *C*. The four genes have been given the symbols *Nx*, *Na*, *Nb* and *Nc*. Each of them is inherited in autotetraploid fashion and each has usually been found in the simplex condition. The genes controlling field immunity from viruses *X* and *C*, however, have been found in the duplex condition in the varieties Cardinal and President, respectively.

The availability of the four genes for use in breeding may be judged from their distribution among potato varieties. *Nx* has been found in 38 of 219 varieties, *Na* in 97 of 190 varieties, *Nb* in 71 of 154 varieties and *Nc* in 75 of 154 varieties. The equality in frequency of the dominant and recessive allelomorphs in the *A*, *B* and *C* series and the marked low frequency of *Nx* are distinctive features of this distribution. Since all the genes are inherited in similar fashion the reason for the marked low frequency of *Nx* is not readily accounted for but its effect is all too plainly evident in the preponderance of susceptible varieties among those in general cultivation. Only five of these are field immune from virus *X*, namely Arran Crest, Epicure, Ninety-fold, King Edward, and the new variety Craigs Defiance.

A comparison between these varieties as a group and other varieties having no protection against virus *X* brings clearly into focus the ubiquity of virus *X* and at the same time demonstrates the value of field immunity as a form of virus control in seed production. In 1937 and 1938 the proportions of each group of varieties granted Stock Seed, Grade A and Grade H certificates* were as follows:

		Acres in each group			Percentage in each group		
Year	Variety group	Stock seed	Grade A	Grade H	Stock seed	Grade A	Grade H
1937	Field immune	541	8296	3640	4·04	61·88	27·15
	Others	191	4340	35040	0·42	9·19	77·55
1938	Field immune	655	10235	1928	4·83	75·48	14·21
	Others	421	8833	36040	0·83	17·53	71·51

These data provide clear evidence that in normal agricultural practice and in areas environmentally suited to seed production, varieties field immune from virus *X* can be maintained at a higher standard of health than varieties lacking this characteristic. This is made possible first by the absence of all diseases of which virus *X* is the sole cause or a component and, secondly, by the ease with which other diseases are observed and removed in these circumstances. Thus field immunity from virus *X* brings about a more effective control of the aphis-borne leaf-roll and *Y* viruses, a factor of the greatest importance in limiting the internal and more dangerous sources of infection in stocks destined for areas where aphis-borne viruses are readily disseminated.

Leaf-roll resistance

Varietal differences towards leaf-roll were examined recently by Loughnane (1941) in Ireland. In field trials he established differences in tolerance, as measured by reduction in vigour and yield, and in susceptibility to infection, as measured by the rate at which the virus infiltrated into stocks. The trials carried out at the Scottish Plant Breeding Station have confirmed the Irish results and, through the wider selection of varieties, have extended the observations. Thus three varieties have been found which have completely withstood intensive conditions of infection both in the field and in the laboratory over a 7-year period. The possibility that they are carriers has been examined but as yet there has been no recovery of virus from any plant tested.

The nature of the resistance appears to be of the same order as that affecting the rate of infiltration of virus into stocks. Evidence leading to this conclusion has been derived from progeny tests. By comparing progenies in diallel series, of the type resistant x a range of susceptibles and susceptible x the same range of susceptibles, it was found that although all the seedlings in each progeny were susceptible, the period required to complete infection was greater in the progenies of resistant parentage than in the corresponding progenies of susceptible parentage. Thus, for example, although seedlings derived from crosses between Shamrock (resistant) and the susceptible varieties Abundance, Up-to-Date, President, Catriona, Golden Wonder, Dunbar Cavalier, British Queen and Pepo were all susceptible, a period of 4 or 5 years under exposure to infection in the field was required to reveal their total susceptibility. Progenies derived from the crosses between Pepo and the same range of susceptibles, on the other hand, became totally infected in 2–3 years' exposure.

* In the years quoted the standards of grading were: Stock seed: 99·95% pure with no disease other than negligible mottle. Grade A: 99·5% pure with not more than 1% in all of mild mosaic, severe mosaic, leaf-roll and wildings. Grade H: 99·5% pure with not more than 3% in all of severe diseases. Mild mosaic was ignored in this grade.

Shamrock would appear, therefore, to possess factors which prevent infection when aggregated and which delay infection when dispersed.

A similar series of crosses between resistant varieties and the varieties Epicure, Kerr's Pink, Claymore, Katahdin, Di Vernon and the seedling 70 (13) have resulted in a small number of seedlings in each progeny which have withstood both exposure in the field and sprout infection. The same range of varieties when crossed with President, Pepo, Alness and Up-to-Date, however, have yielded seedlings all of which are susceptible. These results indicate that the varieties Epicure, etc., possess some factors for resistance in the dispersed form.

Self- and cross-fertilization within the small group of resistant plants has given progenies with varying proportions of resistant and susceptible seedlings. Thus Shamrock selfed has given 66 % resistants and Shamrock crossed with a resistant seedling, 45 % resistants. Other progenies of this type are still segregating. Self- and cross-fertilizations between fully susceptible varieties have given entirely susceptible progenies all of which succumb rapidly in the field. The relatively resistant varieties show varied behaviour when selfed and intercrossed. Thus Katahdin when selfed and when crossed with Di Vernon and 70 (13) produces only susceptibles. Great Scot and Kerr's Pink, on the other hand, when crossed with 70 (13) give a very small number of resistants, and Majestic selfed has yielded one resistant seedling in a small progeny of fifty-eight plants.

These results, though entirely inadequate for full genetic interpretation, at least indicate that within cultivated varieties there are hereditary factors controlling resistance to leaf-roll and that these are several in number and are cumulative in their effect. The utilization of these factors in practical potato breeding is, however, not a matter of direct application. As the results of breeding have shown, the factors are widely dispersed and their resistance value is almost entirely dissipated in out-breeding. By close inbreeding within the small group of varieties and seedlings showing resistance, however, it should be possible to accumulate resistant factors in sufficient quantity to make them available for use in breeding for commercial purposes.

Resistance in wild species

The possibilities of virus resistance are not confined to those found in the cultivated potato. There is, indeed, a much wider field for exploration in the 'wild' material of the American continent. Several reports of resistance from these sources have already been made. Salaman (1921), for instance, found resistance to leaf-roll in the species *S. edinense*. More recently, American workers (Schultz *et al.* 1934) reported a positive resistance to virus *X* in the seedling 41956 which is derived from a native cultivated variety of the island of Chiloe. The expression of this resistance has been shown to be dependent upon the presence of two dominant and complementary genes which are inherited in autotetraploid fashion (Stevenson *et al.* 1939). The diploid species *S. Rybinii* has frequently been described as virus-resistant. Russian workers have mentioned virus resistance in general terms in their investigation of the wild species but, in a recent report (Kameraz, 1940), it was stated that almost all the tuber-bearing species suffer from degeneration when grown near Leningrad. A few, however, were said to suffer only slightly from attack and *S. maglia* remained entirely free.

A preliminary examination of some of the 'wild' material now available in this country has been made. Tests for original virus content have revealed the presence of some virus or other, most frequently virus *X*, in many species and varieties. Three viruses, or rather presumed viruses, which appear to be as yet undescribed have also been found. Their properties of causing top necrosis on Di Vernon and Arran Banner and a necrosis on Irish Chieftain, respectively, serve to differentiate them from our common viruses.

The genes determining top necrosis upon infection with viruses *X*, *A*, *B* and *C* have been found to be well distributed over the species examined. Thus *Nx* has been found in twenty-six clones of five species, *S. tuberosum*, *S. andigenum*, *S. Parodii*, *S. curtilobum*, and *S. Juzepczukii*; *Na* in forty-five clones of ten species, *S. ajuscoense*, *S. chacoense*, *S. demissum*, *S. leptostigma*, *S. longipedicellatum*, *S. Salamanii*, *S. simplicifolium*, *S. infundibuliforme*, *S. andigenum* and *S. curtilobum*; *Nb*, which has proved to be most rare, in thirteen clones of only two species, *S. andigenum* and *S. curtilobum*; and *Nc* in sixteen clones of three species, *S. demissum*, *S. simplicifolium* and *S. andigenum*. Successful infection of some clones has not yet been accomplished. For example, in several trials in which *X*-bearing scions have been grafted on *S. Rybinii* and on several varieties of *S. andigenum* and *S. curtilobum* no symptoms have been observed nor has the virus been recovered. It may well be that positive factors for resistance to virus *X* are present within these clones.

The trials with the leaf-roll virus and virus *Y* are still insufficiently far advanced to speak of the results obtained in terms of resistance. Most clones have shown symptoms of infection with virus *Y*, however, though a few have so far failed to accept the virus either through graft or aphis feeding. Very violent reactions to graft infection with this virus have occurred occasionally, notably on *S. simplicifolium* and a variety of *S. demissum*.

A preliminary examination has also been made of the possibility that resistance to aphis-borne viruses may be determined through resistance to aphis attack. The only observation of interest to record at present is that although *S. polyadenium* appears to

be exceedingly distasteful to aphides it has been infected with aphis-borne virus *Y*.

Conclusion

Although the cultivated potato is the natural host of a number of viruses only four of these, *X*, *A*, *Y* and leaf-roll, appear to be of major significance in the causation of severe disease in this country. Indication has been obtained that some form of resistance, or in the case of virus *Y* a high degree of tolerance, to each of these viruses exists within cultivated varieties of the potato and that each form is controlled by heritable factors. The possibilities of even more useful forms of resistance in the 'wild' material are gradually coming to light and there is considerable prospect, therefore, of achieving a fair measure of control over virus diseases through the introduction of potato varieties which are more resistant than those at present in cultivation. It is not suggested that the elimination of all viruses and virus diseases from potato crops is foreshadowed, nor yet that highly resistant varieties are to be available immediately. On the contrary, for potato breeding for virus resistance is yet in its infancy and a complete understanding of the many issues involved has not yet been reached. The foundations have been laid, however, and there is every reason to anticipate that the difficulties encountered are not insurmountable.

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